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Original Article

Comparative *in vitro* Evaluation of Chemical and Herbal Antimicrobial Agents for Surface Decontamination: A Pharmaceutical Perspective

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ABSTRACT

Background: Maintenance of asepsis is essential for successful endodontic therapy. Gutta-percha cones are susceptible to microbial contamination during handling and storage, and their thermoplastic nature precludes heat sterilization, making rapid chemical disinfection necessary. **Objectives:** This study evaluated the comparative antimicrobial activity of chemical and herbal agents used for rapid gutta-percha cone decontamination against *E. faecalis*. **Materials and Methods:** This *in vitro* experimental study included 80 standardized GP cones (6% taper, size 40), contaminated with *E. faecalis*. The samples were randomly divided into two groups: Group I (chemical disinfectants) comprising 5% sodium hypochlorite (Subgroup IA) and 2% chlorhexidine (Subgroup IB), and Group II (herbal disinfectants) comprising *Aloe vera* extract (Subgroup IIA) and Neem extract (Subgroup IIB). Antimicrobial efficacy was assessed using agar diffusion methods on blood agar and Mueller-Hinton agar by measuring the zones of inhibition. Data were statistically analyzed using Student's *t*-test with a significance level set at $p<0.05$. **Results:** Among chemical disinfectants, 2% chlorhexidine demonstrated the highest antimicrobial activity, with mean zones of inhibition of 24.70±7.26 mm (blood agar) and 27.90±6.23 mm (Mueller-Hinton agar). Among herbal agents, *Aloe vera* showed superior efficacy compared to Neem, with mean inhibition zones of 20.10±6.66 mm and 22.50±8.53 mm, respectively. Intergroup comparisons revealed statistically significant differences between several subgroups ($p<0.05$). **Conclusion:** 2% chlorhexidine was the most effective chemical disinfectant for rapid GP cone decontamination. Herbal agents, particularly *Aloe vera*, exhibited significant antimicrobial activity, indicating their potential as safe and effective chairside alternatives in endodontic practice.

Keywords: *Aloe vera*, Chlorhexidine, Disinfection, *Enterococcus faecalis*, Gutta-percha, Neem, Sodium hypochlorite.

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INTRODUCTION

Successful endodontic treatment relies on effective chemomechanical preparation to eliminate or significantly reduce microorganisms from the root canal system. Strict aseptic protocols must be followed during this procedure to prevent contamination of instruments and obturating materials, thereby avoiding cross-infection (Voroshilov *et al*., 2025).

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Gutta-Percha (GP) is the most widely used obturating material in endodontics. It is a dried coagulated extract obtained from plants of the *Palaquium* genus (family *Sapotaceae*), introduced to dentistry in 1847 (Bansal *et al*., 2020). Even when supplied in sealed packages, gutta-percha cones can acquire contamination during storage and chairside handling, resulting in loss of aseptic integrity (Bansal *et al*., 2020; Ramesh Chandra *et al*., 2017). Conventional sterilization methods using moist or dry heat cannot be employed due to the thermoplastic nature of GP, which may cause structural alterations (Ramesh *et al*., 2017). Therefore, rapid chairside chemical disinfection of GP cones prior to obturation is essential (Voroshilov *et al*., 2025).

Commonly used chemical disinfectants include sodium hypochlorite, chlorhexidine, ethyl alcohol, glutaraldehyde, and formocresol, with disinfection times ranging from 3 to 25 min (Ramesh *et al*., 2017). *Enterococcus faecalis* is frequently linked to refractory endodontic infections and treatment failure because of its resilience under unfavorable intracanal conditions (Liu *et al*., 2024).

Sodium hypochlorite and chlorhexidine are widely used for GP disinfection; however, sodium hypochlorite may alter the surface structure of GP cones and interfere with sealer adhesion, leading to microleakage. Chlorhexidine exhibits broad-spectrum antimicrobial activity and substantivity but also has limitations (Shailja *et al*., 2018). Herbal agents such as *Aloe vera* and neem have gained attention as eco-friendly alternatives. *Aloe vera* possesses antibacterial properties attributed to polysaccharides, anthraquinones, and saponins. Neem (*Azadirachta indica*) contains azadirachtin, which exhibits antimicrobial and anti-adherence properties (Makade *et al*., 2017; Kunjar and Shrivastav, 2022).

Despite their promising properties, evidence supporting the use of herbal agents for rapid chairside disinfection of gutta-percha cones remains limited. Hence, the present study was conducted to evaluate and compare the antimicrobial effectiveness of conventional chemical disinfectants with herbal alternatives, including *Aloe vera* and neem, for rapid gutta-percha cone decontamination.

MATERIALS AND METHODS

Following approval from the Institutional Ethics Committee (MDC_KT_D202010), this *in vitro* experimental study was carried out in the Department of Pedodontics and Preventive Dentistry, in collaboration with the Departments of Microbiology, Biochemistry, and Pharmacology.

Sample Size Calculation

The sample size was calculated based on previously reported antimicrobial studies evaluating disinfectant efficacy against *Enterococcus faecalis*. Assuming a minimum clinically significant difference of 5 mm in the mean zone of inhibition between groups, with an estimated standard deviation of 7 mm, a power of 80%, and a significance level (α) of 0.05, the minimum required sample size was determined to be 18 specimens per subgroup. To compensate for potential experimental variability, 20 gutta-percha cones were included in each subgroup, resulting in a total sample size of 80 specimens.

Eighty standardized gutta-percha cones (6% taper, size 40) were included. *Enterococcus faecalis* was selected as the test organism. Culture media used were brain-heart infusion broth, blood agar, and Mueller-Hinton agar. The disinfecting agents evaluated were 5% sodium hypochlorite, 2% chlorhexidine, *Aloe vera* extract, and neem extract. Ethanol served as the extraction solvent for herbal preparations.

Freeze-dried *E. faecalis* obtained from the Department of Microbiology was reactivated in brain-heart infusion broth. Sterile test tubes containing 5 mL of activated inoculum were incubated at 37ºC for 30 min. Aseptically retrieved gutta-percha cones were immersed in the inoculum and incubated under identical conditions to achieve uniform microbial contamination.

The contaminated cones were randomly assigned into two equal groups (n=40).

Group I (Chemical disinfectants)

- **Subgroup IA:** 5% sodium hypochlorite (n=20).
- **Subgroup IB:** 2% chlorhexidine (n=20).

Group II (Herbal disinfectants)

- **Subgroup IIA:** *Aloe vera* extract (n=20).
- **Subgroup IIB:** Neem extract (n=20).

Fresh *Aloe vera* leaves were washed, the gel was collected, oven-dried, powdered, and subjected to ethanol extraction, followed by filtration and solvent evaporation (Shailja, Ramesh, Anubha, and Shazia, 2018). Neem leaves were dried, pulverized, and extracted using ethanol in a 1:10 weight-to-volume ratio (Natasha *et al*., 2015).

Antimicrobial efficacy against *E. faecalis* was assessed using blood agar and Mueller-Hinton agar diffusion methods. Overnight cultures were spread onto agar plates, and standardized wells were prepared. Each well received 0.1 mL of the respective disinfectant, followed by incubation at 37ºC for 24 hr. The antibacterial effect was quantified by measuring the diameter of inhibition zones.

All observations were systematically recorded and subjected to statistical evaluation using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics (mean and standard deviation) were calculated. Intergroup comparisons were performed using One-Way Analysis of Variance (ANOVA) separately for each culture medium. *Post hoc* pairwise comparisons were conducted using Tukey's test. Confidence interval was set at 95%. p value <0.05 was considered statistically significant.

RESULTS

The mean zone of inhibition (in mm) of the four subgroups against *E. faecalis* is presented in Table 1. Among chemical agents (Group I), subgroup IB demonstrated a greater antibacterial effect than subgroup IA. The mean zone of inhibition for subgroup IB was 24.70±7.26 mm in Blood Agar (BA) and 27.90±6.23 mm in Mueller-Hinton Agar (MHA). Among herbal alternatives (Group II), subgroup IIA showed the highest mean zone of inhibition, with values of 20.10±6.66 mm in BA and 22.50±8.53 mm in MHA, which were greater than those observed for subgroup IIB.

Table 1: Zone of inhibition (mean±standard deviation) of two medium and four groups.

Group	Subgroup	Agent	*n*	Blood Agar (Mean±SD)	Mueller-Hinton Agar (Mean±SD)	% Mean Change	*p* value
Group I	IA	5% NaOCl	20	17.00±6.09	18.70±6.00	10.00	0.54
	IB	2% CHX	20	24.70±7.26	27.90±6.23	12.96	0.31
Group II	IIA	Aloe vera	20	20.10±6.66	22.50±8.53	11.94	0.50
	IIB	Neem	20	13.80±3.74	15.90±6.26	15.22	0.37

(BA - Blood agar, MHA - Muller Hinton agar).

Table 2: *Post hoc* Tukey test for pairwise comparison of mean zone of inhibition (*p* values).

Subgroups	BA medium	MHA medium
Subgroup IA versus Subgroup IB	0.019*	0.003*
Subgroup IIA versus subgroup IIB	0.018*	0.064
Subgroup IA versus Subgroup IIA	0.292	0.264
Subgroup IB versus Subgroup IIB	<0.001*	0.001*
Subgroup IA versus Subgroup IIB	0.174	0.321
Subgroup IB versus Subgroup IIA	0.157	0.123

(BA - Blood agar, MHA - Muller Hinton agar, * - *p* value significant). Abbreviations GP: Gutta-percha; BA: Blood agar; MHA: Mueller-Hinton agar; ANOVA: analysis of variance; NaOCl: sodium hypochlorite; CHX: chlorhexidine.

Post hoc Tukey analysis revealed that subgroup IB demonstrated significantly greater antibacterial activity compared to subgroup IA and subgroup IIB in both culture media ($p<0.05$). A statistically significant difference was also observed between subgroup IIA and subgroup IIB in blood agar ($p=0.018$), whereas no significant difference was noted in Mueller-Hinton agar ($p=0.064$) (Table 2).

DISCUSSION

The persistence of microbial infection within the root canal system and periradicular tissues remains the most significant cause of endodontic failure (Gutmann and Manjarrés, 2018). Endodontic failure is multifactorial, often attributed to anatomical variations, technical errors, biological factors, and iatrogenic complications. Despite high overall success rates, absolute predictability cannot be ensured, and failures may occur before, during, or after treatment (Gulabivala and Ng, 2023). Additional complications such as nerve paresthesia, tissue emphysema, and aspiration or ingestion of instruments have also been reported (Athiban *et al.*, 2012).

Among the microorganisms associated with endodontic failure, *staphylococcus species* are frequently identified as contaminants of Gutta-Percha (GP) cones during storage and handling, underscoring the need for their disinfection (Shetty *et al.*, 2023). *Enterococcus faecalis* is commonly isolated from failed endodontic cases, with prevalence ranging from 24% to 77%. Its ability to survive harsh conditions, resist intracanal medicaments, and form biofilms contributes significantly to treatment failure (Fiore *et al.*, 2019).

Since GP is heat-sensitive and cannot be sterilized using conventional thermal methods without altering its physical properties, chemical disinfection prior to obturation is essential (Kalai *et al.*, 2020). The success of endodontic therapy is closely related to effective microbial elimination before obturation, and prevention of exogenous contamination is equally critical (Bansal *et al.*, 2020). GP remains the most widely used obturating material due to its biocompatibility, dimensional stability, radiopacity, and minimal toxicity (Vishwanath and Rao, 2019). However, clinical handling and exposure significantly increase the risk of microbial contamination, potentially leading to reinfection (Agrawal *et al.*, 2020).

Despite this risk, many clinicians do not routinely disinfect GP cones prior to obturation, highlighting the importance of rapid chairside disinfection followed by aseptic handling (Jain *et al.*, 2019). Rapid sterilization techniques, such as those proposed by Senia *et al.*, remain simple, economical, and reliable (Frank and Pelleu, 1983). Chemical disinfectants are preferred, but the choice of agent is crucial, as certain disinfectants may adversely affect GP surface characteristics (Bansal *et al.*, 2020). Sodium hypochlorite, commonly used at concentrations between 0.5% and 5%, exhibits potent antimicrobial activity but may compromise GP integrity at higher concentrations (Kulkarni and Desai, 2019). Chlorhexidine has been proposed as a safer alternative, demonstrating broad-spectrum antimicrobial activity and substantivity without damaging GP cones (Kalai *et al.*, 2020).

In recent years, herbal agents have gained attention as eco-friendly alternatives. *Aloe vera* and neem (*Azadirachta indica*) extracts exhibit significant antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, and *E. faecalis* (Shenoi *et al.*, 2014; Athiban *et al.*, 2012; Kulkarni and Desai, 2019). Neem leaf extracts, rich in azadirachtin and tannins, demonstrate broad-spectrum antimicrobial efficacy, including effective decontamination of GP cones (Devi and Sharma, 2023). Studies further confirm that ethanolic extracts of neem and *Aloe vera*, alone or in combination, are effective in disinfecting GP cones (Natasha *et al.*, 2015).

While these findings are promising, most studies are *in vitro* and do not fully replicate the clinical oral environment involving saliva, biofilm interactions, and dentinal tubule architecture. Additionally, the effects of herbal disinfectants on GP cone integrity remain underexplored. Future research should include *in vivo* validation, testing against polymicrobial biofilms, and assessment of long-term antimicrobial substantivity. Standardization of concentrations, exposure times, and formulations of herbal extracts will be essential to establish clinically applicable chairside disinfection protocols.

CONCLUSION

Chlorhexidine was found to be an effective chemical disinfectant for rapid decontamination of gutta-percha cones. Herbal agents also demonstrated significant antimicrobial activity, with *Aloe vera* showing the highest efficacy within a short contact time. Neem extract exhibited promising results, supporting the potential of herbal alternatives in endodontic practice. These findings suggest that both chemical and herbal disinfectants can be reliably used for chairside sterilization of gutta-percha cones.

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ABBREVIATIONS

GP: Gutta-percha; **BA:** Blood agar; **MHA:** Mueller-Hinton agar; **ANOVA:** Analysis of variance; **NaOCl:** Sodium hypochlorite; **CHX:** Chlorhexidine.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS CONTRIBUTIONS

A - Research concept and design, B - Collection and/or assembly of data, C - Data analysis and interpretation, D - Writing the article, E - Critical revision of the article, F - Final approval of the article. **Preethi Vem: A-F; Sathwika Rao Nadipelli: A-F; Daneswari V: A-F; Vaishnavi Julakanti: A-F; Shivani Thoom: A-D, F; Deekshita KV: A-D; Naseemoon Shaik: A-F.**

SUMMARY

This *in vitro* study evaluated the antimicrobial efficacy of chemical and herbal agents used for gutta-percha cone decontamination. Among chemical agents, 2% chlorhexidine demonstrated the highest antimicrobial activity against *Enterococcus faecalis*. Among herbal agents, *Aloe vera* showed superior efficacy compared to neem. The findings suggest that while chemical disinfectants remain highly effective, herbal alternatives possess promising antimicrobial potential and may serve as safe, eco-friendly chairside disinfectants.

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