Comparative Evaluation of the Antimicrobial Efficacy of Selected Herbal Preparations and 5.2% Sodium Hypochlorite against *Enterococcus faecalis:* An in Vitro Original Research Study

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ABSTRACT

Background

Effective microbial control is an essential component of successful endodontic treatment. Although cleaning and shaping and the use of antimicrobial agents substantially reduce the bacterial load within the root canal system, residual microorganisms may persist and contribute to reinfection. The potential adverse effects and safety concerns associated with some synthetic antimicrobial agents have stimulated interest in plant-derived alternatives.

Aim

The present in-vitro study aimed to qualitatively evaluate the antimicrobial efficacy of four herbal preparations—*Mimusopselengi* (Bakula), *Azadirachta indica* (Nimba), *Acacia arabica* (Babul), and *Syzygium aromaticum* (Lavanga)—and compare their activity with 5.2% sodium hypochlorite against *Enterococcus faecalis*.

Materials and Methods

Four raw herbal drugs comprising the bark of *Mimusopselengi*, bark of *Azadirachta indica*, bark of *Acacia arabica*, and flowers of *Syzygium aromaticum* were selected. Concentrated herbal preparations were prepared using the decoction method. A

standard strain of *E. faecalis* was subcultured and used to contaminate cleaned extracted teeth. The teeth were autoclaved at 121°C and 15 psi for 15 minutes and subsequently maintained in *E. faecalis* Brain Heart Infusion broth for 21 days. The broth was changed every 7 days while maintaining turbidity equivalent to a 0.5 McFarland standard, approximately 1.5 × 10^8 CFU/mL. Each test solution was applied to one tooth for 15 minutes. Following exposure, the tooth surface was sampled using a sterile swab and inoculated onto one-quarter of a Petri dish. Bacterial growth was assessed after 24 hours of incubation.

Results

The herbal preparations demonstrated variable antimicrobial activity against *E. faecalis*. No bacterial growth was observed following exposure to *Syzygium aromaticum*. The 5.2% sodium hypochlorite group demonstrated fair growth. *Acacia arabica* and *Azadirachta indica* demonstrated moderate growth, whereas *Mimusopselengi* demonstrated heavy growth.

Conclusion

Within the limitations of this qualitative preliminary investigation, *Syzygium aromaticum* demonstrated the greatest apparent antimicrobial activity among the tested herbal preparations and showed no detectable growth following the experimental exposure period. The findings suggest that *S. aromaticum* warrants further investigation as a potential herbal endodontic irrigant. However, larger controlled studies using standardized preparations, adequate replication, quantitative microbiological assessment, and appropriate statistical analysis are required before clinical application can be recommended.

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Keywords:*Enterococcus faecalis*; herbal medicine; herbal irrigant; endodontics; sodium hypochlorite; *Syzygium aromaticum*; *Azadirachta indica*; *Acacia arabica*; *Mimusopselengi*.

INTRODUCTION

The primary objective of endodontic treatment is the effective elimination or reduction of microorganisms from the root canal system and the prevention of subsequent microbial recolonization. Cleaning and shaping constitute fundamental components of root canal therapy; however, the complex anatomy of the root canal system may prevent complete mechanical elimination of microorganisms. Consequently, antimicrobial medicaments and root canal irrigants play an important role in reducing the microbial burden.

Despite adequate chemomechanical preparation, microorganisms may remain within areas that are difficult to access mechanically and may subsequently multiply and contribute to persistent infection or reinfection. The source presentation emphasizes this problem, noting that residual bacteria may remain after cleaning and shaping and subsequently contribute to reinfection.

Enterococcus faecalis is an organism of particular interest in endodontic microbiology. Its association with persistent endodontic infection and treatment failure has been extensively discussed. Love proposed mechanisms by which *E. faecalis* may contribute to endodontic failure, making the organism a commonly investigated target in experimental studies of endodontic antimicrobial agents.

Sodium hypochlorite is one of the most commonly used antimicrobial irrigating agents in endodontic practice. Nevertheless, concerns regarding the potential adverse effects and safety of synthetic chemical agents have stimulated interest in alternative antimicrobial substances. The source presentation specifically proposes that herbal alternatives may offer potential advantages in endodontic applications.

Medicinal plants contain a variety of biologically active constituents and have traditionally been used for antimicrobial and therapeutic purposes. Herbal preparations may therefore represent a potential source of novel antimicrobial agents for endodontic applications.

The present study was undertaken to qualitatively evaluate the antimicrobial activity of four selected herbal preparations—*Mimusopselengi* (Bakula), *Azadirachta indica* (Nimba), *Acacia arabica* (Babul), and *Syzygium aromaticum* (Lavanga)—against *E. faecalis*, and to compare their apparent activity with 5.2% sodium hypochlorite.

AIM

The aim of this study was to evaluate the antimicrobial efficacy of selected herbal preparations and 5.2% sodium hypochlorite against *Enterococcus faecalis* in an in-vitro extracted-tooth model.

Objectives

The objectives were:

1. To prepare selected herbal drugs using the decoction method.
2. To establish *E. faecalis* contamination in extracted teeth.
3. To evaluate the antimicrobial activity of *Mimusopselengi*, *Azadirachta indica*, *Acacia arabica*, and *Syzygium aromaticum*.
4. To compare the observed antimicrobial activity of the herbal preparations with 5.2% sodium hypochlorite.
5. To identify the herbal preparation demonstrating the greatest apparent antimicrobial activity under the experimental conditions.

MATERIALS AND METHODS

Study design

This was a qualitative, preliminary in-vitro experimental study designed to compare the antimicrobial activity of selected herbal preparations with 5.2% sodium hypochlorite against *E. faecalis*.

Test substances

The following test substances were evaluated:

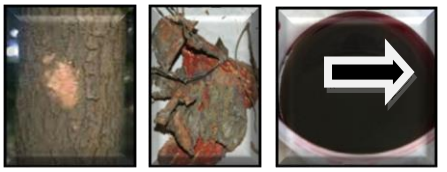
Group	Test substance	Plant part
Herbal group 1	*Mimusopselengi* (Bakula)	Bark
Herbal group 2	*Azadirachta indica* (Nimba)	Bark
Herbal group 3	*Acacia arabica* (Babul)	Bark
Herbal group 4	*Syzygium aromaticum* (Lavanga)	Flower
Chemical comparator	5.2% sodium hypochlorite	-------

The selection of the herbal materials and the concentration of sodium hypochlorite were based on the experimental protocol presented in the source PowerPoint.

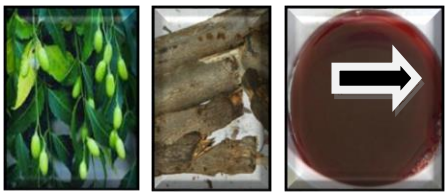
Preparation of herbal preparations

The four raw herbal drugs were processed into concentrated preparations using the **decoction method**. The source presentation describes decoction as the process by which the raw herbal drugs were converted into their concentrated essence or liquor.

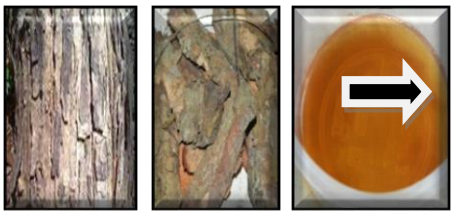
The presentation does not provide sufficient information regarding the precise weight-to-volume ratio, extraction temperature, extraction duration, final concentration, or storage conditions of the individual preparations. These details should therefore be incorporated from the original laboratory protocol before manuscript submission.



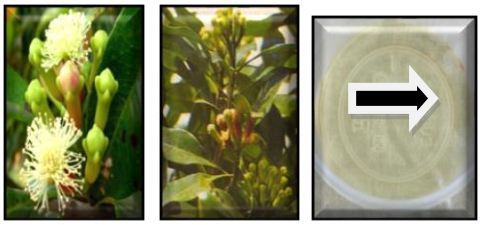
Mimusopselengi(Bakula)
Bark



Azadirachta indica(Nimba)
Bark



Acacia arabica(Babul)
Bark

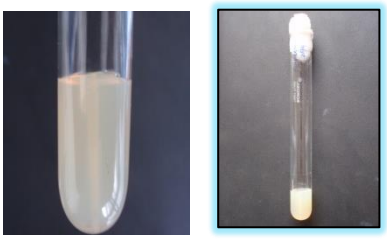


Sygygium aromaticum(Lavanga)
Flower

5.2% Sodium hypochlorite

Bacterial strain

A standard strain of *Enterococcus faecalis* was used for the study. The bacterial culture was maintained in a suitable medium and subsequently subcultured in Bile Esculin Agar prior to use. The presentation specifically documents 24-hour subculture of the *E. faecalis* standard strain in Bile Esculin Agar medium.



Enterococcus faecalis bacteria standard stain preserved in soft agar medium

Preparation of extracted teeth

Cleaned extracted teeth were sterilized by autoclaving at 121°C and 15 psi for 15 minutes. Following sterilization, the teeth were transferred to *E. faecalis*-containing Brain Heart Infusion broth.

Establishment of bacterial contamination

The sterilized extracted teeth were maintained in *E. faecalis* Brain Heart Infusion broth for 21 days. During this period, the broth was changed every 7 days. The bacterial turbidity was maintained equivalent to a 0.5 McFarland standard, corresponding to approximately 1.5 × 10^8 CFU/mL according to the study protocol.

The available presentation does not provide sufficient information to determine whether the bacterial colonization was quantitatively confirmed within dentinal tubules or whether a standardized biofilm quantification procedure was performed.

Exposure to test solutions

Following the bacterial contamination period, the experimental teeth were exposed to the respective test solutions.

According to the study protocol, **one tooth was placed in each solution for 15 minutes**.

The tested groups consisted of:

- *Mimusopselengi*
- *Azadirachta indica*
- *Acacia arabica*
- *Syzygium aromaticum*
- 5.2% sodium hypochlorite

Microbiological assessment

After exposure to the respective test solutions, the surface of the tooth was sampled using a sterile swab. The swab was rubbed over the tooth surface and the recovered material was streaked over one-quarter of a Petri dish.

The inoculated plates were incubated for 24 hours, after which bacterial colony formation was evaluated. The results were interpreted qualitatively according to the degree of bacterial growth.

Because the experimental presentation describes the investigation as qualitative, the findings were categorized as no growth, fair growth, moderate growth, or heavy growth rather than subjected to inferential statistical analysis.

RESULTS

The experimental groups demonstrated different degrees of bacterial growth after the 15-minute exposure period.

No bacterial growth was observed in the *Syzygium aromaticum* group. The 5.2% sodium hypochlorite group demonstrated fair growth. *Acacia arabica* and *Azadirachta indica* demonstrated moderate growth, while *Mimusopselengi* demonstrated heavy bacterial

growth. These observations are directly reported in the study presentation.

Table 1. Qualitative assessment of bacterial growth following exposure to the test substances

Test substance	Qualitative bacterial growth
Syzygium aromaticum (Lavanga)	No growth
5.2% sodium hypochlorite	Fair growth
Acacia arabica (Babul)	Moderate growth
Azadirachta indica (Nimba)	Moderate growth
Mimusopselengi (Bakula)	Heavy growth

Based on the qualitative observations, *Syzygium aromaticum* demonstrated the greatest apparent antimicrobial activity among the tested herbal preparations.

The qualitative order of apparent antimicrobial activity was:

Syzygium aromaticum > 5.2% sodium hypochlorite > Acacia arabica ≈ Azadirachta indica >Mimusopselengi

This ranking is descriptive only and should not be interpreted as a statistically validated hierarchy because the source study does not report replication or inferential statistical analysis.

DISCUSSION

The elimination of microorganisms from the root canal system remains a central objective of endodontic treatment. Although mechanical preparation can substantially reduce bacterial contamination, microorganisms may persist in areas that are inaccessible to instrumentation. The use of antimicrobial irrigants is therefore an important component of root canal disinfection.

The present qualitative investigation evaluated four herbal preparations against *E. faecalis* and compared their observed activity with 5.2% sodium hypochlorite. A marked variation in bacterial growth was observed among the experimental groups.

The most notable finding was the absence of detectable bacterial growth in the *Syzygium aromaticum* group following the 15-minute exposure period. In contrast, the sodium hypochlorite group demonstrated fair growth, while *Acacia arabica* and *Azadirachta indica* demonstrated moderate growth and *Mimusopselengi* demonstrated heavy growth.

The observed activity of *S. aromaticum* is of particular interest because clove has traditionally been used for its medicinal properties. The study presentation therefore identifies *S. aromaticum* as the herbal preparation with the most promising antimicrobial effect in the experimental model.

However, the present findings need to be interpreted cautiously. The study was explicitly qualitative, and the available protocol indicates that one tooth was used in each solution. Thus, the experiment does not provide sufficient replication to establish whether the observed differences represent reproducible differences in antimicrobial efficacy.

The activity observed with 5.2% sodium hypochlorite provides an important comparator. Sodium hypochlorite remains a standard antimicrobial agent in endodontic practice. The present finding that *S. aromaticum* produced no detectable growth whereas sodium hypochlorite produced fair growth is potentially interesting; however, it cannot by itself establish that the herbal preparation is superior to sodium hypochlorite. Differences in preparation, concentration, contact conditions, bacterial recovery, and experimental variability could influence the outcome.

The results for *Acacia arabica* and *Azadirachta indica* were intermediate, with moderate bacterial growth. These findings suggest that their preparations had some apparent antimicrobial effect under the experimental conditions but were less effective than *S. aromaticum*.

Mimusopselengi* demonstrated heavy bacterial growth and consequently showed the least apparent antibacterial activity among the four herbal preparations tested. This finding highlights the importance of experimentally evaluating individual plant preparations rather than assuming that all herbal products possess equivalent antimicrobial properties.

Previous literature has investigated herbal alternatives against *E. faecalis*. Prabhakar et al. evaluated herbal alternatives including Triphala and green tea polyphenols and compared them with conventional endodontic antimicrobial agents. The study cited in the source presentation provides a relevant precedent for evaluating plant-derived antimicrobial agents against *E. faecalis* biofilms.

The present study contributes preliminary observations concerning four additional herbal preparations. Nevertheless, direct comparison with other published studies should be undertaken cautiously because antimicrobial efficacy can be influenced by plant species, plant part, extraction method, concentration, bacterial strain, biofilm maturity, exposure time, and method of bacterial assessment.

CLINICAL RELEVANCE

The findings raise the possibility that selected herbal preparations may have potential applications as antimicrobial agents in endodontics. In particular, the absence of detectable bacterial growth following exposure to *S. aromaticum* suggests that this plant preparation merits further investigation.

The source presentation proposes several potential advantages of herbal agents, including antimicrobial activity, availability, cost-effectiveness, shelf life, low toxicity, and potentially reduced microbial resistance.

However, these proposed advantages were **not directly evaluated in the present experiment**. Therefore, they should be regarded as potential considerations rather than demonstrated outcomes of this study.

Before any herbal preparation can be recommended as a root canal irrigant, its antimicrobial activity must be evaluated alongside other clinically relevant properties, including cytotoxicity, tissue compatibility, dentin interaction, penetration into dentinal tubules, smear-layer effects, stability, physicochemical properties, and compatibility with other endodontic materials.

.CONCLUSION

Within the limitations of this qualitative in-vitro investigation, the selected herbal preparations demonstrated variable antimicrobial activity against *Enterococcus faecalis*.

Among the tested herbal preparations, *Syzygium aromaticum* (Lavanga) demonstrated the most favorable result, with no detectable bacterial growth following the experimental exposure period. 5.2% sodium hypochlorite demonstrated fair bacterial growth, while *Acacia arabica* and *Azadirachta indica* demonstrated moderate growth and *Mimusopselengi* demonstrated heavy growth.

The findings suggest that *Syzygium aromaticum* may represent a promising candidate for further investigation as a potential herbal endodontic irrigant. Nevertheless, the present study does not provide sufficient evidence to recommend its clinical substitution for sodium hypochlorite. Rigorous, adequately powered, quantitative and controlled studies are required to establish its antimicrobial efficacy, safety, and clinical applicability.

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