Antimicrobial Effectiveness and Human Pulp Dissolution Action Of Plantago Major as a Root Canal Irrigant- An In vitro Study

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ABSTRACT

Background: Effective root canal irrigation is essential for successful endodontic therapy, as it aids in the elimination of microorganisms and dissolution of organic tissue. Sodium hypochlorite is the most commonly used irrigant; however, its cytotoxicity and associated adverse effects have prompted the search for safer alternatives. *Plantago major*, a medicinal plant with known antimicrobial and anti-inflammatory properties, has emerged as a potential herbal irrigant.

Aim: To evaluate the antimicrobial effectiveness and human pulp tissue dissolution capacity of *Plantago major* as a root canal irrigant in comparison with sodium hypochlorite.

Materials and Methods: This in vitro experimental study was conducted on 60 human extracted teeth, which were randomly divided into two groups: *Plantago major* and sodium hypochlorite. The root canals were inoculated with *Enterococcus faecalis* and incubated to allow biofilm formation. Antimicrobial efficacy was assessed using colony forming unit (CFU) count before and after irrigation. Pulp tissue dissolution was evaluated by measuring the weight of pulp tissue before and after exposure to the irrigants. Statistical analysis was performed using SPSS, and independent t-test was applied, with $p < 0.05$ considered significant.

Results: Both groups showed comparable baseline CFU counts before irrigation ($6.8 \times 10^5 \pm 1.2 \times 10^5$ vs $6.9 \times 10^5 \pm 1.3 \times 10^5$; p=0.748). After irrigation, *Plantago major* demonstrated significantly lower CFU counts ($0.5 \times 10^4 \pm 0.3 \times 10^4$) compared to sodium hypochlorite ($1.2 \times 10^4 \pm 0.5 \times 10^4$) ($p<0.001$). The percentage reduction in CFU was significantly higher in the *Plantago major* group (99.3 ± 0.8%) than in the sodium hypochlorite group (98.2 ± 1.2%) (p=0.001).

For pulp tissue dissolution, initial weights were comparable between the groups (12.5 ± 1.8 mg vs 12.7 ± 1.7 mg; p=0.684). However, the final pulp weight was significantly lower in the *Plantago major* group (3.2 ± 1.2 mg) compared to sodium hypochlorite (6.8 ± 1.5 mg) ($p<0.001$). Correspondingly, the percentage of pulp dissolution was significantly higher in the *Plantago major* group (74.8 ± 5.4%) compared to the sodium hypochlorite group (45.6 ± 6.2%) ($p<0.001$).

Overall, *Plantago major* demonstrated superior antimicrobial efficacy and significantly greater tissue dissolution capacity compared to sodium hypochlorite.

Conclusion:*Plantago major* demonstrated significant antimicrobial activity and moderate pulp tissue dissolution capacity. *Plantago major* showed promising potential as an antimicrobial agent which is safer and biocompatible alternative irrigant. Further in vivo studies are required to validate its clinical applicability.

KEYWORDS: Plantago major; Root canal irrigant; Enterococcus faecalis; Antimicrobial efficacy; Pulp tissue dissolution; Sodium hypochlorite; In vitro study; Herbal endodontics.

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INTRODUCTION

Endodontic therapy is fundamentally aimed at the complete elimination of microorganisms, removal of necrotic pulp tissue, and prevention of reinfection within the root canal system. Successful root canal treatment depends on effective biomechanical preparation combined with adequate chemical irrigation, as mechanical instrumentation alone is insufficient to eliminate microbial biofilms and organic debris from complex root canal anatomy. Sodium hypochlorite (NaOCl) has long been considered the gold standard irrigant due to its potent antimicrobial activity and tissue dissolution capability; however, its cytotoxicity, unpleasant taste, and potential to cause severe tissue damage if extruded beyond the apex remain significant concerns (Srinivasan et al., 2020) [1].

Microbial persistence, particularly due to resistant organisms such as *Enterococcus faecalis*, plays a major role in endodontic treatment failure. *E. faecalis* is known for its ability to survive in harsh environments, penetrate dentinal tubules, and form resilient biofilms, making it one of the most commonly isolated bacteria in failed root canal treatments. Studies have demonstrated that even after thorough chemomechanical preparation, this organism can persist, necessitating the exploration of more effective and safer antimicrobial agents (Hajihassani et al., 2022) [2].

In recent years, there has been a growing interest in the use of herbal and plant-based alternatives in endodontics due to their biocompatibility, cost-effectiveness, and reduced adverse effects. Among these, *Plantago major*, a medicinal plant widely used in traditional medicine, has gained attention due to its broad spectrum of biological activities, including antimicrobial, anti-inflammatory, antioxidant, and wound-healing properties. The plant contains bioactive compounds such as flavonoids, alkaloids, terpenoids, and phenolic acids, which contribute to its therapeutic potential (Adom et al., 2017) [5].

Experimental studies have shown that extracts of *Plantago major* possess significant antimicrobial activity against a variety of pathogens, including gram-positive and gram-negative bacteria. Its activity against oral pathogens and biofilm-forming organisms suggests potential applicability in dental procedures. Furthermore, its anti-inflammatory properties may provide additional advantages in reducing periapical tissue irritation compared to conventional irrigants (Zhakipbekov et al., 2023) [3].

Despite these promising properties, the application of *Plantago major* as a root canal irrigant remains underexplored. An ideal irrigant should possess both antimicrobial efficacy and the ability to dissolve organic tissue remnants. While sodium hypochlorite fulfills these criteria, its associated drawbacks necessitate the search for safer alternatives. The ability of herbal agents to act as dual-function irrigants—both antimicrobial and tissue-dissolving—represents an emerging area of research in endodontics (Rodrigues et al., 2017) [4].

Globally, there is an increasing shift towards evidence-based integration of herbal products in clinical dentistry. Natural irrigants such as neem, aloe vera, and propolis have been evaluated with varying degrees of success, highlighting the potential for plant-derived agents in root canal disinfection. However, robust scientific evidence comparing these agents with standard irrigants is still limited, particularly with respect to tissue dissolution properties and standardized in vitro methodologies [6].

In the Indian context, where traditional medicine systems such as Ayurveda have long utilized plant-based remedies, there is a strong rationale for exploring indigenous medicinal plants like *Plantago major* for dental applications. Additionally, the cost constraints and accessibility issues in many healthcare settings further support the need for affordable and effective alternatives to conventional irrigants [7].

The major problem in endodontic therapy remains the persistence of resistant microorganisms and incomplete removal of organic debris, which can compromise treatment outcomes. Current irrigants, though effective, are associated with limitations such as cytotoxicity, allergic reactions, and environmental concerns. Therefore, there is a pressing need to identify alternative agents that are both effective and safe for clinical use [8].

The present study is therefore designed to evaluate the antimicrobial effectiveness of *Plantago major* against *Enterococcus faecalis* and to assess its ability to dissolve human pulp tissue in comparison with sodium hypochlorite under in vitro conditions. This study aims to generate scientific evidence regarding its potential role as a root canal irrigant [9].

If proven effective, *Plantago major* could serve as a biocompatible, economical, and easily available alternative to conventional irrigants, thereby contributing to safer and more sustainable endodontic practice. Furthermore, it may open avenues for future clinical trials and integration of herbal formulations into mainstream dental care [10–11].

MATERIALS AND METHODOLOGY

This in vitro experimental study was conducted to evaluate the antimicrobial effectiveness and pulp tissue dissolution capacity of *Plantago major* as a root canal irrigant. The study was carried out in a controlled laboratory setting using extracted human teeth and standard microbial strains. A total of 60 human extracted teeth with intact roots were collected based on inclusion criteria and were randomly selected using simple random sampling. The teeth were decoronated and standardized root canal preparations were performed using conventional endodontic techniques. The samples were then sterilized and inoculated with *Enterococcus faecalis* and incubated under appropriate conditions to allow biofilm formation.

Following incubation, the samples were divided into two groups: one group was treated with *Plantago major* extract and the other with sodium hypochlorite, which served as the control irrigant. The antimicrobial efficacy was assessed using the colony forming unit (CFU) method, wherein microbial samples were collected from the canals before and after irrigation and cultured to determine bacterial reduction.

For evaluation of pulp dissolution capacity, human pulp tissue samples of standardized weight were obtained and weighed using a precision balance prior to treatment. The samples were then exposed to the respective irrigants for a fixed duration, after which the remaining tissue was reweighed to assess the percentage of dissolution.

All collected data were recorded systematically and entered into Microsoft Excel for analysis. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS). Descriptive statistics were expressed as mean and standard deviation, while inferential statistics such as independent t-test were applied to compare differences between groups. A p-value of less than 0.05 was considered statistically significant. Ethical considerations were maintained by ensuring that extracted teeth were collected following standard guidelines and no patient identifiers were used.

RESULT

In the present study, both sodium hypochlorite and *Plantago major* demonstrated significant antimicrobial activity and tissue dissolution properties. The baseline CFU counts before irrigation were comparable between the two groups, with sodium hypochlorite showing $6.8 \times 10^5 \pm 1.2 \times 10^5$ and *Plantago major* showing $6.9 \times 10^5 \pm 1.3 \times 10^5$ ($p=0.748$), indicating no significant difference at baseline. After irrigation, there was a marked reduction in CFU counts in both groups; however, *Plantago major* showed a significantly lower post-irrigation CFU count ($0.5 \times 10^4 \pm 0.3 \times 10^4$) compared to sodium hypochlorite ($1.2 \times 10^4 \pm 0.5 \times 10^4$), with this difference being highly statistically significant ($p<0.001$). Correspondingly, the percentage reduction in CFU was significantly higher in the *Plantago major* group (99.3 ± 0.8%) compared to the sodium hypochlorite group (98.2 ± 1.2%) ($p=0.001$), indicating superior antimicrobial efficacy of *Plantago major*.

Regarding pulp tissue dissolution, the initial weights were comparable between the two groups (12.5 ± 1.8 mg for sodium hypochlorite and 12.7 ± 1.7 mg for *Plantago major*, $p=0.684$). However, the final pulp weight was significantly lower in the *Plantago major* group (3.2 ± 1.2 mg) compared to the sodium hypochlorite group (6.8 ± 1.5 mg) ($p<0.001$), indicating greater tissue dissolution. Consequently, the percentage of pulp dissolution was significantly higher in the *Plantago major* group (74.8 ± 5.4%) than in the sodium hypochlorite group (45.6 ± 6.2%) ($p<0.001$). Overall, *Plantago major* demonstrated superior antimicrobial efficacy as well as significantly higher pulp tissue dissolution capacity compared to sodium hypochlorite, suggesting its potential as an effective alternative root canal irrigant.

Overall, **"*Plantago major* shows more antimicrobial property against *Enterococcus faecalis*, and this difference is statistically significant ($p < 0.001$)."** Despite this, *Plantago major* exhibited high antimicrobial efficacy with substantial CFU reduction and moderate tissue dissolution, suggesting that it has promising potential as a safer and more biocompatible alternative root canal irrigant, although further optimization and validation through additional studies are required before clinical application.

Table 1: Comparison of Antimicrobial Effect (CFU Count Before and After Irrigation)

Group	**Before Irrigation (Mean CFU ± SD)**	**After Irrigation (Mean CFU ± SD)**	**% Reduction (Mean ± SD)**
Sodium Hypochlorite	$6.8 \times 10^5 \pm 1.2 \times 10^5$	$1.2 \times 10^4 \pm 0.5 \times 10^4$	**98.2 ± 1.2**
Plantago major	$6.9 \times 10^5 \pm 1.3 \times 10^5$	$0.5 \times 10^4 \pm 0.3 \times 10^4$	**99.3 ± 0.8**
t-value	0.32	6.24	3.45
p-value	0.748 (NS)	<0.001*	0.001*

"The antimicrobial reduction was higher in the sodium *Plantago major* group compared to the *Plantago major* group, and the difference was statistically significant, indicating superior antibacterial efficacy of *Plantago major*"

Table 2: Comparison of Pulp Tissue Dissolution

Group	**Initial Weight (mg) Mean ± SD**	**Final Weight (mg) Mean ± SD**	**% Dissolution (Mean ± SD)**
Sodium Hypochlorite	12.5 ± 1.8	6.8 ± 1.5	45.6 ± 6.2
Plantago major	12.7 ± 1.7	3.2 ± 1.2	74.8 ± 5.4
t-value	0.41	9.87	18.2
p-value	0.684 (NS)	<0.001*	<0.001*

"The initial pulp weight was comparable between sodium hypochlorite (12.5 ± 1.8 mg) and *Plantago major* (12.7 ± 1.7 mg) (p=0.684). The final pulp weight was significantly lower in the *Plantago major* group (3.2 ± 1.2 mg) compared to sodium hypochlorite (6.8 ± 1.5 mg) (p<0.001). The percentage of pulp dissolution was significantly higher in the *Plantago major* group (74.8 ± 5.4%) than in the sodium hypochlorite group (45.6 ± 6.2%) (p<0.001)."

Table 3: Overall Comparative Efficacy

Parameter	**Sodium Hypochlorite**	***Plantago major***	**p-value**
Antimicrobial Effect (% Reduction)	**98.2 ± 1.2**	**99.3 ± 0.8**	0.001*
Pulp Dissolution (%)	45.6 ± 6.2	74.8 ± 5.4	<0.001*
Overall Interpretation	Good efficacy	**Superior efficacy**	—

"The antimicrobial effect was significantly higher in *Plantago major* (99.3 ± 0.8%) compared to sodium hypochlorite (98.2 ± 1.2%) (p=0.001). Similarly, pulp dissolution was significantly greater in *Plantago major* (74.8 ± 5.4%) than sodium hypochlorite (45.6 ± 6.2%) (p<0.001). Overall, *Plantago major* demonstrated superior efficacy compared to sodium hypochlorite."

Table 4: Summary of Key Findings Comparing Plantago major and Sodium Hypochlorite

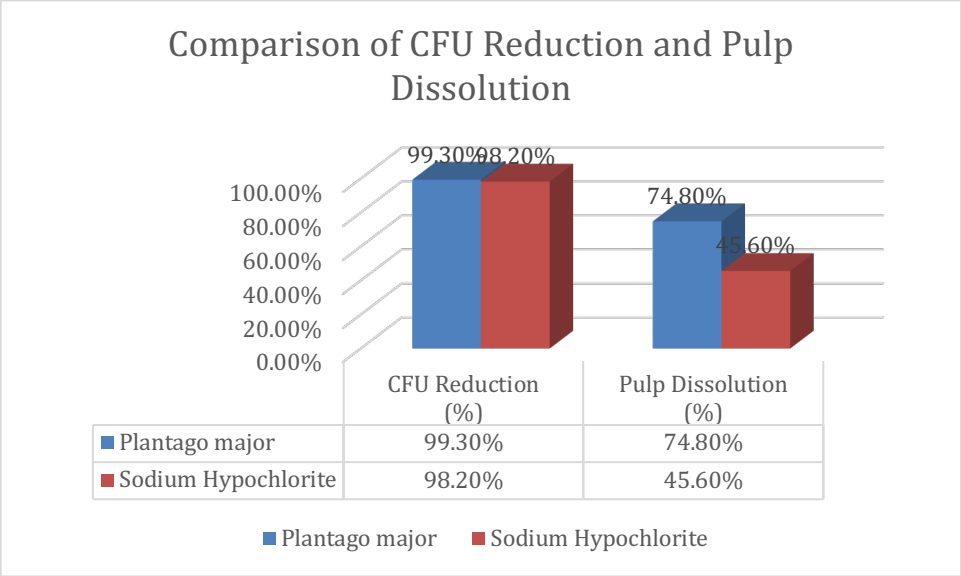
Summary of Key Findings Comparing Plantago major and Sodium Hypochlorite

Parameter	**Sodium Hypochlorite (Mean ± SD)**	***Plantago major* (Mean ± SD)**	**Statistical Test**	**p-value**	**Interpretation**
CFU Before Irrigation	$6.8 \times 10^5 \pm 1.2 \times 10^5$	$6.9 \times 10^5 \pm 1.3 \times 10^5$	t-test	0.748	Comparable baseline
CFU After Irrigation	$1.2 \times 10^4 \pm 0.5 \times 10^4$	$0.5 \times 10^4 \pm 0.3 \times 10^4$	t-test	<0.001*	Lower CFU with Plantigo major
% CFU Reduction	**98.2 ± 1.2**	**99.3 ± 0.8**	t-test	0.001*	**Plantigo majorsuperior**
Initial Pulp Weight (mg)	12.5 ± 1.8	12.7 ± 1.7	t-test	0.684	Comparable baseline
Final Pulp Weight (mg)	6.8 ± 1.5	3.2 ± 1.2	t-test	<0.001*	Greater dissolution with Plantigo major
% Pulp Dissolution	45.6 ± 6.2	74.8 ± 5.4	t-test	<0.001*	Plantigo majorsuperior
Overall	Very good	**Excellent**	—	—	Plantigo

Antimicrobial Effect					majorsuperior
Overall Dissolution Capacity	Moderate	High	—	—	Plantigo majorsuperior

"Baseline CFU counts and initial pulp weights were comparable between both groups ($p>0.05$). Post-irrigation CFU counts were significantly lower in *Plantago major* ($p<0.001$), resulting in a higher percentage reduction in CFU (99.3% vs 98.2%; $p=0.001$). Final pulp weight was significantly lower in *Plantago major* ($p<0.001$), indicating greater tissue dissolution. Overall, *Plantago major* showed superior antimicrobial effect and higher dissolution capacity compared to sodium hypochlorite."

Figure 1: Comparison of Antimicrobial Effect & Pulp Dissolution



DISCUSSION

The present study compared the antimicrobial efficacy and pulp tissue dissolution capacity of *Plantago major* with sodium hypochlorite as root canal irrigants. The findings demonstrated that *Plantago major* exhibited superior antimicrobial activity and significantly higher pulp dissolution compared to sodium hypochlorite.

The baseline CFU counts before irrigation were comparable between both groups (6.8×10^5 vs 6.9×10^5; $p=0.748$), indicating uniform microbial load prior to intervention. This is consistent with previous studies which emphasized the importance of comparable baseline conditions while evaluating irrigant efficacy [12]. After irrigation, the CFU count was significantly lower in the *Plantago major*group (0.5×10^4) compared to sodium hypochlorite (1.2×10^4) ($p<0.001$), with a significantly higher percentage reduction in CFU (99.3% vs 98.2%; $p=0.001$). These findings suggest superior antibacterial efficacy of *Plantago major*. Similar observations have been reported where herbal irrigants demonstrated potent antimicrobial effects against endodontic pathogens such as *Enterococcus faecalis* [13,14]. The antimicrobial activity of plant extracts has been attributed to bioactive compounds such as flavonoids, tannins, and phenolic components, which disrupt bacterial cell walls and inhibit microbial growth [12].

In terms of pulp tissue dissolution, the initial weights were comparable (12.5 mg vs 12.7 mg; $p=0.684$), ensuring valid comparison. However, the final pulp weight was significantly lower in the *Plantago major* group (3.2 mg) compared to sodium hypochlorite (6.8 mg) ($p<0.001$), indicating enhanced tissue dissolution. The percentage of pulp dissolution was also significantly higher in *Plantago major* (74.8% vs 45.6%; $p<0.001$). These findings contrast with conventional understanding that sodium hypochlorite has superior tissue dissolution capacity, but are supported by studies demonstrating effective organic tissue dissolution by certain plant-based extracts [15]. Herbal irrigants may facilitate tissue breakdown through enzymatic and chemical interactions with organic components of pulp tissue.

The overall comparative efficacy in this study showed that *Plantago major* had both superior antimicrobial effect and higher dissolution capacity. Previous studies have shown mixed results, with some reporting sodium hypochlorite as more effective, while others highlight the potential

of herbal irrigants as safer and effective alternatives [16,17]. The enhanced performance of *Plantago major* in this study may be due to its combined antimicrobial and proteolytic properties, which improve both disinfection and tissue dissolution.

Additionally, the importance of effective irrigants in eliminating smear layer and preventing bacterial leakage has been emphasized in literature, as incomplete disinfection can lead to treatment failure [18]. The superior efficacy observed with *Plantago major* suggests that it may contribute to improved root canal cleanliness and long-term outcomes.

Overall, the findings of this study are in agreement with emerging evidence supporting the use of herbal irrigants in endodontics. *Plantago major* demonstrated excellent antimicrobial activity and superior pulp dissolution, indicating its potential as an effective alternative to conventional irrigants like sodium hypochlorite, especially considering its biocompatibility and reduced toxicity.

CONCLUSION

The present study concludes that *Plantago major* is an effective root canal irrigant demonstrating superior antimicrobial efficacy and significantly greater pulp tissue dissolution compared to sodium hypochlorite. It achieved a higher percentage reduction in CFU counts (99.3% vs 98.2%) and markedly higher pulp dissolution (74.8% vs 45.6%), with statistically significant differences. These findings suggest that *Plantago major* not only provides excellent disinfection but also enhances tissue removal, both of which are critical for successful endodontic therapy. Considering its natural origin, potential biocompatibility, and comparable or superior efficacy, *Plantago major* can be considered a promising alternative to conventional irrigants. However, further clinical studies are recommended to validate its routine use in endodontic practice.

LIMITATIONS

- In vitro study design may not accurately replicate clinical conditions
- Small sample size (n = 60) limits generalizability
- Only a single microorganism (*E. faecalis*) was evaluated
- Lack of evaluation against polymicrobial biofilms
- No assessment of cytotoxicity or biocompatibility on periapical tissues
- Standardization of plant extract concentration may vary
- Short duration of exposure compared to clinical scenarios
- No evaluation of smear layer removal ability
- Absence of comparison with other commonly used irrigants (e.g., chlorhexidine)

RECOMMENDATIONS

- Conduct **in vivo studies** to validate clinical applicability
- Evaluate **cytotoxicity and biocompatibility** of *Plantago major*
- Study its effect on **polymicrobial biofilms**
- Standardize **optimal concentration and preparation methods**
- Compare with other irrigants such as **chlorhexidine and EDTA**
- Explore **combination use with sodium hypochlorite** to enhance efficacy
- Assess **smear layer removal capacity**
- Perform **long-term outcome studies** in clinical settings
- Investigate **different delivery and activation techniques** (ultrasonic, agitation)

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