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Efficacy of herbal intracanal medicaments in endodontic disinfection

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Abstract:
Persistent root canal infections caused by *Enterococcus faecalis* remain a major challenge in endodontic therapy, necessitating effective and biocompatible intracanal medicaments. Therefore, it is of interest to evaluate the antimicrobial efficacy of neem extract, propolis extract, curcumin extract and calcium hydroxide in 100 patients randomly allocated into 4 groups of 25 participants each. All medicaments significantly reduced intracanal bacterial counts after 7 days, with calcium hydroxide showing 85.8% reduction, followed by neem extract (85.2%), propolis extract (81.8%) and curcumin extract (78.0%), while no clinically significant difference was observed between neem and calcium hydroxide. Neem extract showed antimicrobial efficacy comparable to calcium hydroxide, whereas propolis and curcumin also exhibited substantial antibacterial activity against *Enterococcus faecalis.* Thus, data support herbal intracanal medicaments, particularly neem extract, as promising biocompatible alternatives or adjuncts for enhancing root canal disinfection and improving endodontic treatment outcomes.

Keyword: Endodontics, herbal intracanal medicaments, neem, propolis, curcumin, calcium hydroxide, root canal disinfection, *enterococcus faecalis*

Background:
The key to successful endodontic treatment is to eliminate all microorganisms from the root canal system and to prevent reinfection. Inflammatory reactions begin when microbes invade the pulp and root canal space and progress to pulpal necrosis and periapical disease **[1]**. Despite the development of instrumentation and irrigation techniques, complete disinfection of the root canal system is still challenging due to the complexity of the root canal system, lateral canals, isthmuses, apical ramifications and dentinal tubules. One of the most important factors contributing to persistent periapical infection and endodontic treatment failure is the presence of residual microorganisms which survive chemomechanical preparation **[2].** Between treatment visits, therefore, intracanal medicaments are used to decrease the number of residual bacteria and enhance treatment results. Calcium hydroxide has been considered the gold standard intracanal medicament due to its high alkaline pH, antimicrobial properties and hard tissue promoting activity **[3]**. However, some studies have reported that some resistant microorganisms such as *Enterococcus faecalis* can remain in the canals treated with calcium hydroxide and cause persistent infections **[4].** This restriction has led to the development of alternative or adjunctive intracanal medicaments that have greater antimicrobial activity and biocompatibility. The popularity of natural and herbal products in modern endodontics has been growing due to their wide spectrum antimicrobial activity, low toxicity, anti-inflammatory activity and low potential for microbial resistance. Initial studies on natural medicaments revealed their possible uses in the dental field and proposed that plant-based compounds could be used as alternatives to chemical compounds **[5, 6]**. Over the past few years, more and more scientific evidence has been found to support the use of herbal preparations for root canal disinfection. *Azadirachta indica* (neem) is one of the most widely studied herbal remedies for endodontics. It is rich in biologically active compounds like nimbidin, azadirachtin and quercetin which have strong antibacterial, antifungal, anti-inflammatory and antioxidant properties. Neem extract has been shown to have potent antimicrobial properties against *E. faecalis* and other endodontic pathogens, which makes it a potential intracanal medicament **[7].** Likewise, propolis a natural resinous material made by honeybees, is rich in flavonoids, phenolic acids and aromatic compounds, which are responsible for its antimicrobial and tissue-healing properties. Earlier studies have demonstrated that propolis has antimicrobial activity and can break down bacterial biofilms in infected root canals **[8].** Turmeric *(Curcuma longa)* has also been of interest due to the antimicrobial, antioxidant and anti-inflammatory properties of its main bioactive compound, curcumin. Experimental research has shown that curcumin can be very effective at decreasing endodontic pathogens and inhibit biofilm formation, but may be dependent on concentration and delivery methods **[9]**. In addition to antimicrobial activity, herbal medicaments can also play a role in tissue repair and modulation of inflammatory

responses, both of which are crucial to successful endodontic healing **[10]**. Herbal medicines have been highlighted in recent reviews as a potential field of study in endodontics due to their beneficial biological properties and clinical applications **[11]**. Moreover, randomized clinical trials testing the efficacy of herbal intracanal medicaments have shown promising antimicrobial results similar to those of conventional intracanal medicaments **[12]**. Therefore, it is of interest to assess and compare the antimicrobial activity of neem, propolis and curcumin as herbal intracanal medicaments and to compare the effectiveness of these herbal intracanal medicaments with calcium hydroxide in reducing intracanal bacterial load and elimination of *Enterococcus faecalis* during endodontic treatment.

Materials and Methodology:

Study design:

A prospective randomized comparative clinical study was conducted in the Department of Conservative Dentistry and Endodontics of a tertiary dental care teaching institution.

Study duration:

The study was conducted over a period of 18 months from January 2024 to June 2025.

Sample size:

A total of 100 patients requiring root canal treatment were included in the study. The Sample Size distribution is given in **Table 1.**

Study population:

Patients presenting with asymptomatic necrotic permanent teeth requiring root canal treatment were screened for eligibility.

Inclusion criteria:

[1] Patients aged 18–60 years.
[2] Single-rooted permanent teeth with necrotic pulp.
[3] Radiographic evidence of chronic apical periodontitis.
[4] Teeth suitable for conventional root canal therapy.
[5] Patients willing to provide written informed consent.

Exclusion criteria:

[1] Patients with systemic diseases affecting healing.
[2] Patients receiving antibiotics within the previous four weeks.
[3] Pregnant or lactating women.
[4] Teeth with root fractures.
[5] Previously treated teeth.
[6] Teeth with advanced periodontal disease.
[7] Patients allergic to herbal products.

Ethical considerations:

Approval was obtained from the Institutional Ethics Committee prior to commencement of the study. Written informed consent was obtained from all participants.

Randomization:

Eligible patients were randomly allocated into four groups using computer-generated random numbers. Allocation concealment was maintained using sealed opaque envelopes.

Clinical procedure:

First visit:

After administration of local anesthesia and rubber dam isolation, access cavity preparation was performed under aseptic conditions. The tooth surface was disinfected using 30% hydrogen peroxide followed by 5% iodine solution before access preparation. Working length determination was performed using an electronic apex locator and confirmed radiographically. Biomechanical preparation was completed using rotary nickel-titanium instruments following a crown-down technique. Irrigation was performed using 3% sodium hypochlorite throughout instrumentation.

Microbial sampling before medication (S1):

Following instrumentation and drying of canals, sterile paper points were inserted into the canal for 60 seconds.

The paper points were transferred into sterile transport media and sent immediately for microbiological analysis.

Placement of intracanal medicaments:

[1] **Group I – Neem Extract:** A standardized neem extract paste was prepared and introduced into the canal using a lentulo spiral.
[2] **Group II – Propolis Extract:** Propolis paste was prepared in a standardized concentration and placed into the canal.
[3] **Group III – Curcumin Extract:** Curcumin gel was introduced into the canal as the intracanal medicament.
[4] **Group IV – Calcium Hydroxide:** Commercial calcium hydroxide paste was used according to manufacturer instructions. The access cavity was sealed using temporary restorative material.

Second visit (after 7 days):

[1] Temporary restoration was removed under rubber dam isolation.
[2] The intracanal medicament was flushed using saline and irrigation protocol.
[3] The canal was dried using sterile paper points.

Post-medication sampling (S2):

[1] Sterile paper points were again inserted into the canal for microbiological sampling.
[2] Samples were transferred immediately for microbiological evaluation.

Microbiological analysis:

Samples were cultured on Brain Heart Infusion agar and selective media for *Enterococcus faecalis*. Colony-forming units (CFU/mL) were counted using standard microbiological techniques.

Percentage reduction in bacterial count was calculated using:

$$Percentage\ reduction = \frac{S1 - S2}{100} \times 100$$

Where,
[1] S1 = Pre-medication bacterial count
[2] S2 = Post-medication bacterial count

Outcome measures:
Primary outcome:
Reduction in intracanal bacterial count (CFU/mL)

Secondary outcomes:
[1] Percentage bacterial reduction.
[2] Elimination of *E. faecalis*.
[3] Incidence of post-operative pain.
[4] Adverse reactions to medicaments.

Statistical analysis:
Data were entered into Microsoft Excel and analyzed using SPSS version 26.0. Mean ± Standard Deviation was calculated for continuous variables. Frequencies and percentages were calculated for categorical variables. Paired t-test was used for intragroup comparison. Chi-square test was used for categorical variables. A p-value <0.05 was considered statistically significant.

Results:
A total of 100 patients requiring root canal treatment were enrolled and randomly allocated into four equal groups: Neem extract (Group I), Propolis extract (Group II), Curcumin extract (Group III) and Calcium Hydroxide (Group IV), with 25 patients in each group **(Table 1)**. The efficacy of the intracanal medicaments was assessed by comparing pre-medication and post-medication bacterial counts and calculating the percentage reduction in colony-forming units (CFU/mL). The presence of *Enterococcus faecalis* after treatment was also evaluated. Baseline bacterial counts were comparable among all groups, indicating homogeneity before treatment. Following seven days of intracanal medication, a statistically significant reduction in bacterial counts was observed in every group (p<0.001). Calcium hydroxide demonstrated the highest mean bacterial reduction (85.8%), closely followed by neem extract (85.2%). Propolis showed an 81.8% reduction, while curcumin exhibited a 78.0% reduction. Despite slight variations, all herbal medicaments achieved substantial antimicrobial effects, approaching those of the conventional medicament **(Table 2).** The majority of patients in the neem and calcium hydroxide groups achieved bacterial reductions exceeding 80%. More than 90% reduction was observed in 44.0% of calcium hydroxide cases and 40.0% of neem-treated cases. Curcumin demonstrated relatively lower efficacy, with only 20.0% of cases achieving reductions greater than 90%. Intergroup comparison revealed a statistically significant difference in percentage bacterial reduction (p=0.041), indicating superior antimicrobial activity of neem and calcium hydroxide compared with curcumin **(Table 3).** All samples showed the presence of *E. faecalis* before intracanal medication. Following treatment, a marked reduction in positive cultures was observed in all groups. Calcium hydroxide showed the highest elimination rate (92.0%), while neem achieved an elimination rate of 88.0%, closely approximating the control group. Propolis eliminated *E. faecalis* in 84.0% of cases, whereas curcumin achieved elimination in 76.0% of patients. The reductions were statistically significant within all groups (p<0.001) **(Table 4).**

Table 1: Sample size distribution

Group	Intracanal Medicament	Sample Size
Group I	Neem Extract	25
Group II	Propolis Extract	25
Group III	Curcumin Extract	25
Group IV	Calcium Hydroxide (Control)	25
Total		100

Table 3: Comparison of percentage reduction in intracanal bacterial load among groups

Percentage Reduction	Neem (n=25)	Propolis (n=25)	Curcumin (n=25)	Calcium Hydroxide (n=25)
>90% reduction	10 (40.0%)	8 (32.0%)	5 (20.0%)	11 (44.0%)
80–90% reduction	9 (36.0%)	10 (40.0%)	8 (32.0%)	9 (36.0%)
70–79% reduction	5 (20.0%)	5 (20.0%)	9 (36.0%)	4 (16.0%)
<70% reduction	1 (4.0%)	2 (8.0%)	3 (12.0%)	1 (4.0%)
Mean Reduction (%)	85.2 ± 6.4	81.8 ± 7.1	78.0 ± 8.3	85.8 ± 5.9
p-value	0.041*			

**Statistically significant*

Table 2: Comparison of mean bacterial counts before and after intracanal medication

Group	Pre-medication CFU (Mean ± SD)	Post-medication CFU (Mean ± SD)	Mean Reduction (%)	p-value
Neem	6.21 ± 0.81	0.92 ± 0.34	85.2%	<0.001*
Propolis	6.15 ± 0.76	1.12 ± 0.39	81.8%	<0.001*
Curcumin	6.18 ± 0.79	1.36 ± 0.42	78.0%	<0.001*
Calcium Hydroxide	6.20 ± 0.83	0.88 ± 0.31	85.8%	<0.001*

**Statistically significant*

Table 4: Elimination of *Enterococcus faecalis* following intracanal medication

Group	Positive Before Treatment n (%)	Positive After Treatment n (%)	Complete Elimination n (%)	p-value
Neem	25 (100%)	3 (12.0%)	22 (88.0%)	<0.001*
Propolis	25 (100%)	4 (16.0%)	21 (84.0%)	<0.001*
Curcumin	25 (100%)	6 (24.0%)	19 (76.0%)	<0.001*
Calcium Hydroxide	25 (100%)	2 (8.0%)	23 (92.0%)	<0.001*

**Statistically significant*

Discussion:

In this study, all intracanal medicaments were effective in reducing the number of bacteria, thus confirming their antimicrobial efficacy. Calcium hydroxide exhibited the highest bacterial reduction, followed closely by neem extract and propolis and curcumin also exhibited significant antibacterial activity. The results of this study corroborate the increasing interest in herbal products as alternative intracanal medicaments. The antimicrobial properties of natural products in dentistry have been known for a long time. Sinha and Sinha **[13]** first pointed out the significance of plant-derived medicaments as biologically compatible alternatives to synthetic chemicals used in dental practice, noting their antimicrobial, anti-inflammatory and antioxidant properties. Later, Venkateshbabu *et al.* reviewed the growing importance of natural therapeutic agents in endodontics and proposed that herbal products may play a significant role in the disinfection of the canal and healing of the tissues due to their various biological properties **[12]**. Neem extract was the most effective herbal agent in the present study and was able to reduce the bacteria to a level similar to calcium hydroxide. Neem has been known for its wide range of antimicrobial properties. Chandrappa *et al.* showed that neem extract exhibited strong inhibitory activity against *Enterococcus faecalis,* one of the most resistant microorganisms associated with chronic endodontic infections **[9]**. The antimicrobial activity of neem is mainly attributed to the presence of bioactive constituents like nimbidin, azadirachtin and quercetin which disrupt the integrity of the cell wall, enzyme systems and biofilm formation in bacteria. The results of the present study corroborate these observations and also support the use of neem as a promising intracanal medicament. In the present study, propolis also exhibited significant antibacterial activity. The antimicrobial activity of propolis has been well documented in the endodontic literature. Zare Jahromi *et al.* reported that propolis has excellent antimicrobial properties against the microorganisms of the root canal due to the presence of flavonoids, phenolic acids and aromatic compounds that disrupt the metabolism and function of microorganisms **[6]**. In addition, propolis has been showed to have anti-inflammatory and tissue healing properties, which render it an interesting material for endodontic use. The results obtained in the present study are thus consistent with previous findings that propolis has therapeutic properties in root canal disinfection. The antimicrobial activity of curcumin was found to be statistically significant, but not as effective as neem and calcium hydroxide. Devaraj *et al.* also investigated the antibiofilm activity of photoactivated curcumin against *E. faecalis* and found that there was significant reduction in the bacteria, but not as much as some of the conventional medicaments **[8]**. The relatively poor performance of curcumin in the present study could be explained by its low aqueous solubility and low ability to penetrate into mature biofilms and dentinal tubules. However, its antimicrobial and anti-inflammatory effects remain to be a promising adjunctive agent in endodontics. The recent advances in regenerative endodontics have further emphasized the need for effective antimicrobial strategies. Ribeiro *et al.* **[14]** highlighted that intracanal medicaments used in successful regenerative procedures must be able to reduce the microbial load and create a favorable environment for the survival of stem cells and tissue regeneration. In this respect, herbal medicines may have a special benefit, due to their better biocompatibility than many synthetic medicines. The growing popularity of herbal intracanal medicaments has also been reinforced by studies on the biological safety of these products. Abdeltawab *et al.* showed that plant-based intracanal medicaments are biocompatible and have good antibacterial activity against endodontic pathogens **[11]**. Likewise, Karobari *et al.* studied the use of herbal drugs in endodontics and found that natural drugs have great antimicrobial, anti-inflammatory and antioxidant properties and have very few side effects **[10]**. These are especially relevant in the context of the potential for cytotoxicity, allergic reactions and microbial resistance with some conventional medicines. Recent studies have further confirmed the efficacy of herbal medicines in endodontics. Palwankar *et al.* showed that herbal products have a significant antimicrobial activity in primary endodontic infections, especially when used along with ultrasonic activation techniques **[2]**. Golla *et al.* also reported that herbal extracts exhibited high antimicrobial activity against *E. faecalis* and could be used as an alternative to the conventional intracanal medicaments **[4]**. The results obtained in the present study are in strong support of these findings. The complexity of root canal infections has been better understood with advances in microbiological research. Sedani *et al.* pointed out the complexity of the microbial ecology found in infected root canal systems and stressed the importance of using multi-spectrum antimicrobial treatment that could disrupt biofilms and resistant bacterial communities **[15]**. Furthermore, Chohan *et al.* showed that better disinfection procedures can result in better antimicrobial results for endodontic pathogens **[16]**. New technologies based on nanotechnology have also proven to be promising. Bukhari *et al.* reported that catalytic nanoparticles have strong antimicrobial activity and could be a new innovation in endodontic disinfection strategies **[17]**. Aldosari *et al.* recently conducted a systematic review that also showed the clinical efficacy of propolis in endodontics and endorsed its use as an adjunctive medicament that can enhance antimicrobial effects and tissue healing **[7]**. The present study yielded encouraging results, but there are some limitations that should be noted. The number of samples was relatively small and only short-term antimicrobial results were assessed. In addition, microbial assessment was performed by culture and may have missed unculturable species. Previous molecular research by Rolph *et al.* and Subiksha *et al.* showed that molecular identification methods can detect a wider range of microorganisms from endodontic infections than traditional culture methods **[18, 19]**. Multi-center clinical studies that include molecular microbiology and long-term clinical outcomes are thus warranted.

Conclusion:

All herbal intracanal medicaments significantly reduced intracanal bacterial load, with neem extract showing

antimicrobial efficacy comparable to calcium hydroxide against *Enterococcus faecalis*. Propolis and curcumin also exhibited substantial antibacterial activity, supporting their potential role in improving root canal disinfection. These findings identify herbal intracanal medicaments as promising biocompatible alternatives or adjuncts to conventional endodontic therapy, warranting further long-term clinical evaluation.

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