



Comparative Evaluation of Antimicrobial Efficacy of Allium Sativum, Glycyrrhiza Glabra and Calcium Hydroxide as an Intracanal Medicament Against Enterococcus Faecalis: An In-Vitro Study

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KEYWORDS

Allium sativum, Glycyrrhiza glabra, calcium hydroxide, intracanal medicament, antimicrobial efficacy.

ABSTRACT:

Context:

Persistent endodontic infections are often linked to Enterococcus faecalis, a resilient microorganism capable of penetrating dentinal tubules and surviving harsh intracanal conditions. Conventional medicaments like calcium hydroxide show limited effectiveness, prompting interest in herbal alternatives.

Aims:

To compare the antimicrobial efficacy of Allium sativum, Glycyrrhiza glabra, and calcium hydroxide against E. faecalis when used as intracanal medicaments.

Methods and Material:

Ninety extracted single-rooted teeth were prepared and inoculated with E. faecalis, then divided into three groups (n=30): Allium sativum, Glycyrrhiza glabra, and calcium hydroxide. After 15 days, CFUs were recorded to assess antimicrobial efficacy. Antibacterial efficacy was evaluated by calculating the percentage reduction in colony count

Statistical analysis used:

Student's unpaired t-test was used for inter- and intragroup comparisons.

Results:

All medicaments showed significant antimicrobial activity. Glycyrrhiza glabra had the highest CFU reduction ($94.80 \pm 1.81\%$), followed by Allium sativum ($83.96 \pm 5.52\%$) and calcium hydroxide ($74.24 \pm 7.22\%$) with significant differences ($p=0.0001$).



Conclusions:

Glycyrrhiza glabra shows superior antimicrobial activity over conventional calcium hydroxide and may serve as an effective intracanal medicament.

INTRODUCTION:

Root canal therapy explain how to cleaning root canals and a pulp chamber infected by bacteria by this extracting infected pulp tissue and doing disinfection of the canal system to lessen the microbial load and help in preventing the recolonization. Failed root canal treatments are commonly infused with bacteria such as *E. faecalis*, because of their ability to form biofilms and resistance to intracanal medicaments^{1,2}.

The use of calcium hydroxide ($\text{Ca}(\text{OH})_2$) has become treating medicament of choice due to its sound Antimicrobial characteristics and creation of the alkaline environment which prevents survival of bacteria. A rise of interest in other intracanal medicaments has expanded inclusion of drugs from plants because of their appeal on resistance of antibiotics and side effects of synthetic medicines^{3,4}.

Garlic or *Allium sativum* exhibit broad and potent overcome antimicrobial characteristics through inhibition of toxin production and expression of an enzyme on Gram positive and Gram negative bacteria. *Glycyrrhiza Glabra* or Liquorice, an herbal plant known in an organic medicine consists of glycyrrhizin which has potent antibacterial activity against *E. Fecalis* and fibroblasts^{5,6}.

In as much as researches have documented with the antibacterial efficacy with *Allium sativum* and *Glycyrrhiza glabra*, not much has been done to compare their effectiveness with that of $\text{Ca}(\text{OH})_2$.

Therefore, the present study was conducted with the aim to compare and evaluate the antimicrobial efficacy of **Allium Sativum**, **Glycyrrhiza Glabra** and **Calcium Hydroxide** as an Intracanal medicament against *Enterococcus Faecalis* in endodontics.

METHODOLOGY

The non-clinical experimental study was conducted in Pravara Institute of Medical Sciences for a time period of 2 years. The Institutional ethical committee gave ethical

approval. The ethical approval number is PIMS/RDC/IEC/UG-PG/ 17-2023⁷.

PREPARATION OF TOOTH SPECIMENS

Ninety Freshly extracted, single rooted single canal teeth (extracted for orthodontic purposes) were selected. The samples were washed with running tap water and cleaned using an ultrasonic scaler. Disinfection was performed using 5.25% sodium hypochlorite (Prime Dental Solutions), followed by autoclave sterilization and further stored in saline until further use⁷.

RANDOMIZATION OF SAMPLES

The collected teeth were randomly divided into three groups using a simple randomization method (**Table no. 1**). Three labeled boxes (1, 2, and 3) were used, and teeth were allocated using a chit-based selection process, ensuring an equal distribution of 30 teeth per group⁷

GROUPS	NUMBER OF TEETH	TYPE OF INTRACANAL MEDICAMENT USED
Group 1	30	Allium Sativum
Group 2	30	Glycyrrhiza Glabra
Group 3	30	Calcium Hydroxide

BIOMECHANICAL PREPARATION OF ROOT CANALS

The coronal portion of all samples was sectioned 2–3 mm below the cemento-enamel junction using a safe-sided diamond disk (SHOFU Dental ASIA-Pacific Pte. Ltd.) to standardize the root length at 10 mm. Root canals were prepared using ProTaper Next rotary files (Dentsply Tulsa Endodontics, USA) from F1, F2 and F3 following the step-down technique. Debridement was completed using 17% EDTA (Dent Wash, Prime Dental Solutions) for 5 minutes, followed by 5.25% sodium hypochlorite (Prime Dental Solutions) for another 5 minutes. All



infection control protocols recommended by the CDC (2003 guidelines) were followed⁷.

PREPARATION OF ALLIUM SATIVUM AND GLYCYRRHIZA GLABRA INTRACANAL MEDICAMENTS

The powdered forms of Allium Sativum (SAI HERBS, powdered form prepared into a paste) and Glycyrrhiza Glabra (FOOD HERBS, powdered form prepared into a paste) were utilized. A total of 100 grams of each powder was boiled in 1 liter of distilled water and reduced to a final volume of 50 ml. Hydroxyethyl cellulose (Akshar chem) was added as a thickening agent in a 2:1 (volume/weight) ratio to achieve a paste-like consistency. The mixtures were stirred using a magnetic stirrer, stored in amber-colored bottles, and transferred to conventional syringes for application. This preparation was conducted at the College of Biosciences and Technology (Pravara Institute of Medical Sciences)⁷.

CONTAMINATION OF ROOT CANALS

All 90 teeth were inoculated with *Enterococcus faecalis* (ATCC 29212). The bacterial colonies were isolated and cultured in 5 ml of brain-heart infusion broth for 4 hours at 37°C. Each sample was contaminated with a 10-ml inoculum of *E. faecalis* and incubated for 21 days. After incubation, dentin scraping was performed using a #30 H file, followed by flooding the canals with sterile saline. Paper points were placed in each canal for 60 seconds and then transferred into sterile test tubes containing 1.0 ml of saline solution. A 10-fold dilution was prepared, and 0.1 ml was plated onto Mueller Hinton Agar medium. Samples were incubated at 37°C for 24 hours, and colony counts were recorded. This procedure was carried out in the Department of Microbiology, Rural Medical College, Loni.

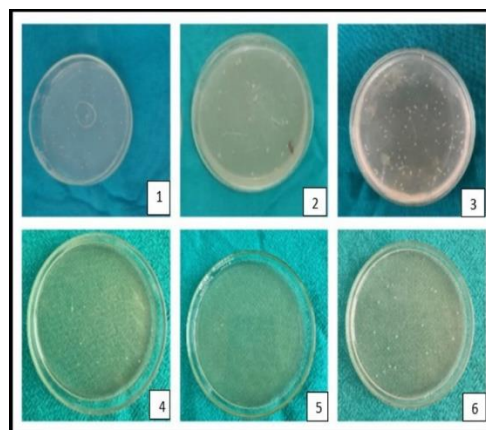
Figure A: Procedural steps



1. Biomechanical preparation.
2. Teeth to be contaminated by *E. Faecalis* in BHI broth.
3. Paper points transferred to test tubes containing 10 fold dilution of saline pre medication.
4. Paper points transferred to test tubes containing 10 fold dilution of saline post medication.
5. Herbal powders used for intracanal medications.
6. Prepared herbal intracanal medicaments stored in amber colored bottles until further use.
7. Intracanal medicaments transferred in syringes.
8. Digital colony counter.

Post-Medication Colony Count Assessment: Intracanal medicaments were injected using a syringe in three groups (n=30 each): Group 1 (Allium sativum), Group 2 (Glycyrrhiza glabra), and Group 3 (Calcium hydroxide). After placement, samples were incubated for 15 days with sterile saline. The medicaments were then removed, dentin was scraped with a #30 H-file, and samples were transferred to test tubes with saline. A 10-fold dilution was prepared, plated on Mueller-Hinton Agar, and incubated. Colony counts were recorded on day 15.

Figure B. *E. Faecalis* colonies on Mueller Hinton Agar.



- 1, 2 and 3 Pre-medication colonies.
4, 5 and 6 Post medication colonies.

Efficacy Calculation:

Antibacterial efficacy was evaluated by calculating the percentage reduction in colony count (%RCC) using the formula:

$$\%RCC = \left(\frac{[\text{Initial} - \text{Final colony count}]}{\text{Initial count}} \right) \times 100.$$

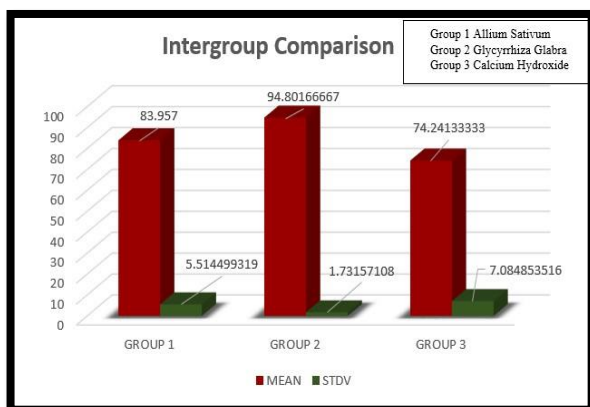
RESULTS AND OBSERVATION



Graph no. 1 Intergroup Comparison of Antimicrobial Efficacy Against *E. faecalis*

(Student's Unpaired t-test – Percentage Reduction in CFU)

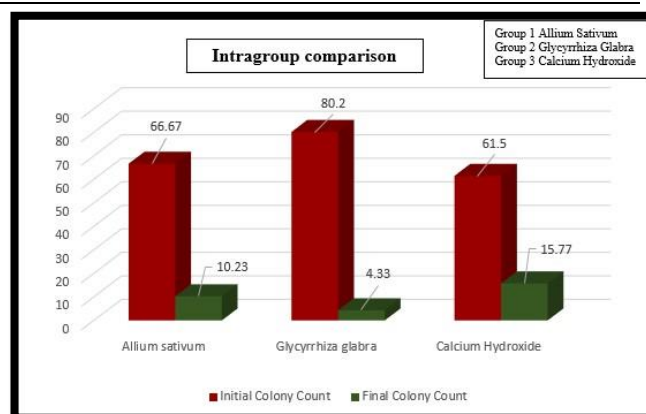
The intergroup comparison of percentage reduction in CFU by Student's unpaired t-test demonstrated statistically significant differences in antimicrobial potency among *Allium sativum*, *Glycyrrhiza glabra*, and Calcium hydroxide. *Glycyrrhiza glabra* exhibited the highest mean percentage reduction ($94.80 \pm 1.81\%$), significantly higher than both *Allium sativum* ($83.96 \pm 5.52\%$, $t=10.222$, t-test demonstrated statistically significant differences in antimicrobial potency $p=0.0001$) and Calcium hydroxide ($74.24 \pm 7.22\%$, $t=15.113$, $p=0.0001$), confirming its greater antimicrobial potency. *Allium sativum* also produced far superior outcomes compared to Calcium hydroxide ($t=5.851$, $p=0.0001$), proving that although the three agents kill *E. faecalis* effectively, *Glycyrrhiza glabra* has the highest killing potential, followed by *Allium sativum* and then Calcium hydroxide.



Graph no. 2 Intragroup Comparison of Antimicrobial Efficacy Against *E. faecalis*

(Student's Unpaired t-test – Reduction in CFU)

The intragroup comparison by Student's unpaired t-test showed statistically significant microbial colony count reductions from initial to final measurements in all three groups. *Allium sativum* had a significant reduction from 66.67 ± 37.68 to 10.23 ± 5.97 ($t=8.113$, $p=0.0001$), which is strong



evidence of its antimicrobial activity. *Glycyrrhiza glabra* too had the maximum reduction, from 80.20 ± 46.74 to 4.33 ± 2.87 ($t=8.872$, $p=0.0001$), and had strong evidence

of antibacterial activity. Calcium hydroxide similarly attained a high decrease from 61.50 ± 35.99 to 15.77 ± 8.83 ($t=6.761$, $p=0.0001$), although the impact was weaker than that of the herbal preparations.

These findings validate that the three agents had a good impact in decreasing microbial colonies, with *Glycyrrhiza glabra* having the best intragroup antimicrobial activity, followed by *Allium sativum* and then Calcium hydroxide.

DISCUSSION

Root canal treatment (RCT) is an essential treatment that eliminates infection from the root canal system, avoids reinfection, and maintains the natural tooth. The success of RCT relies on correct diagnosis, biomechanical preparation, efficient disinfection, and correct obturation, with disinfection being the most important factor for long-term success. Intracanal medicaments augment mechanical and chemical debridement by attacking residual bacteria and facilitating periapical healing. While primary infections tend to be caused by anaerobes such as *Porphyromonas* and *Fusobacterium*, chronic infections are most often caused by *Enterococcus faecalis* because of resistance, biofilm development, and the ability to thrive in adverse conditions^{8,9}.

Effective RCT starts with accurate diagnosis by radiographs, pulp vitality testing, and clinical examinations to diagnose pulpal and periapical disease. Adequate access cavity preparation provides visibility and complete debridement, whereas biomechanical preparation integrates mechanical debridement and chemical disinfection, mostly with sodium hypochlorite (NaOCl), for killing infection. Owing to intricate canal anatomy,



Intracanal medicaments are utilized for prolonged disinfection by sustained release¹⁰.

General irrigants such as NaOCl, chlorhexidine (CHX), and EDTA contribute to microbial control but each with its own limitations necessitating judicious selection to ensure effective root canal disinfection¹¹.

Intracanal medicaments facilitate root canal disinfection, especially in necrotic pulp and chronic apical periodontitis. Siqueira & Lopes (1999) and Estrela & Holland (2003) emphasized the antimicrobial activity of calcium hydroxide but challenged its potential to eliminate endotoxins^{12,13}. Gomes et al. (2003) and Kim et al. (2015) noted that the combination of calcium hydroxide with chlorhexidine enhanced the effect against *E. faecalis*, but Athanassiadis et al. (2007) saw no noteworthy antimicrobial benefit over chlorhexidine alone^{14,15}. Parirokh & Torabinejad (2010) noted less postoperative pain with the combination, and Sahebi et al. (2012) noted calcium hydroxide with silver nanoparticles improved antibacterial effects^{16,17,18}. Yassen et al. (2015), however, cautioned against dentine weakening with long-term exposure, and Martinho & Gomes (2018) raised cytotoxicity issues with periodontal ligament fibroblasts^{19,20}. These studies emphasize its clinical advantages but also continued concerns regarding long-term effects. The need for herbal alternative medicaments arises from concerns over cytotoxicity, antibiotic resistance, and dentin weakening linked to conventional agents.

Practitioners need to evaluate evidence to make the optimum intracanal medicament choice. Herbal solutions, based on Ayurveda, Traditional Chinese Medicine (TCM), and Unani medicine, have attracted scientific interest for endodontic infections. Chauhan et al. (2004) proved the efficacy of neem against *E. faecalis*, and Mohammadi & Abbott (2009) emphasized the requirement for biocompatible alternatives to chlorhexidine^{21,22}. The 2010s have witnessed rising studies on herbal alternatives to calcium hydroxide, and studies in 2023 revealed that curcumin, allicin, gingerol, and cinnamon are effective against *E. faecalis*. The discoveries advocate for the increased use of herbal medicaments in endodontics.

Allium sativum (garlic) is investigated as an intracanal medicament because of its antimicrobial activity, biocompatibility, and possibility as a natural substitute. Research by Bakri & Douglas (2005)

and Octavia (2019) proved its effectiveness against *E. faecalis*,

with increased concentrations proving similar results to 2% chlorhexidine²³. Siddique (2019) discovered that ethanolic garlic-lemon extract had greater antimicrobial activity over time than NaOCl. Nonetheless, cytotoxicity concerns remain, as Devaraju (2024) reported that increased concentrations decrease cell viability, and Mehta (2021) reported it was less effective than NaOCl and diode laser^{24,25}. Omer (2022) reported an antagonistic effect when used with calcium hydroxide²⁶. Although promising, more studies are required to optimize its formulation for endodontic application.

Glycyrrhiza glabra (licorice) has been researched for its anti-bacterial activity against *E. faecalis*, a primary pathogen in chronic endodontic infections. Kriker & Yahia (2013) reported its flavonoid extract showed inhibitory activity²⁷, while Tamhankar (2020) observed a 25% aqueous extract was similarly antibacterial to propolis but greater than calcium hydroxide. Its combination with *Salvia officinalis* was as effective as 0.12% chlorhexidine in a 2023 study²⁸. Yet, Gldas (2016) and Anand (2016) reported that licorice extract (10%) was much less effective than NaOCl, cetrimide, neem, and myrrh against *E. faecalis* biofilms. Although promising, its restricted efficacy is of concern regarding its clinical utility and requirement for further optimization²⁹.

Intracanal medicament formulation influences root canal disinfection through stability, penetration, and antimicrobial activity. Aqueous *Glycyrrhiza glabra* or *Allium sativum* is biocompatible and more diffusible but less retained and stable²⁶. Ethanol-based solutions increase extraction, biofilm penetration, and antibacterial effect but are rapidly evaporative and can soften dentin³⁰.

Gel systems provide extended antimicrobial contact and targeted drug delivery but less penetration of deep tubules⁷. Research indicates *Allium sativum* is superior to *Glycyrrhiza glabra*, particularly in ethanol or gel form. After disinfection, obturation with bioceramic sealers and warm compaction avoids reinfection, and a coronal restoration provides for long-term tooth stability.

The intragroup analysis revealed a statistically significant reduction in colony counts within each group following treatment. *Allium sativum*, *Glycyrrhiza glabra*, and Calcium hydroxide all demonstrated effective antimicrobial action, with *Glycyrrhiza glabra*



showing the most substantial decrease, followed by *Allium sativum* and Calcium hydroxide. This confirms that all three agents successfully reduced microbial loads post-application (**Refer to graph no. 2**).

The intergroup comparisons highlighted that *Glycyrrhiza glabra* was significantly more effective than both *Allium sativum* and Calcium hydroxide, as shown by its higher percentage reduction in CFU. *Allium sativum* also outperformed Calcium hydroxide, reinforcing the superior antimicrobial efficacy of the herbal agents compared to the conventional medicament (**Refer to graph no. 1**).

Recent studies highlight the strong antimicrobial potential of *Glycyrrhiza glabra* and *Allium sativum* against *Enterococcus faecalis*, particularly in reducing bacterial load. Sedighinia et al. (2012) and Birring et al. (2021) reported their efficacy against biofilms^{30,31}, while Mehta et al. (2021) confirmed *A. sativum*'s antibacterial effects, albeit less than NaOCl²⁵. The current study extends these findings by evaluating their sustained action as intracanal medicaments rather than short-term irrigants.

Contradictory evidence exists, such as lower biofilm efficacy of *G. glabra* noted by Gldas et al. (2016), and

A. sativum's lower immediate efficacy compared to NaOCl²⁹. In comparison, *Calcium hydroxide* (Ca(OH)₂) has shown moderate effectiveness, limited by bacterial resistance and penetration depth. Based on current findings, *G. glabra* exhibited the highest antimicrobial efficacy, followed by *A. sativum* and then Ca(OH)₂, highlighting the promise of natural agents as effective alternatives or adjuncts in endodontic disinfection.

CONCLUSION:

This research compared the antimicrobial activity of *Glycyrrhiza glabra*, *Allium sativum*, and Calcium Hydroxide against *Enterococcus faecalis*. *G. glabra* was most effective, followed by *A. sativum*, with Calcium Hydroxide being the least active. The three agents significantly decreased bacterial load, showing the promise of herbal alternatives as intracanal medicaments. More clinical studies are required to replicate these results in practice.

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