

Research Article

## Nigella sativa derived Nano Bioactive Antimicrobial as a Next Generation Intracanal Medicament for Resistant Endodontic Pathogens

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**Abstract:** This study investigated the antimicrobial efficacy of thymoquinone, the active component of *Nigella sativa*, against *Enterococcus faecalis* (ATCC 29212) and compared it with calcium hydroxide. Thymoquinone dissolved in 10% DMSO was evaluated using MIC, MBC, and anti-adherence assays. It showed strong activity, with an MIC of 1.6 µg/ml and an MBC of 3.2 µg/ml. Sub-MIC concentrations also reduced bacterial adherence. These findings suggest thymoquinone is a promising alternative or adjunctive intracanal medicament to calcium hydroxide, supporting further investigation through clinical studies.



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## 1. INTRODUCTION

The primary goal of endodontic therapy is to completely eliminate pathogenic microorganisms from the root canal system. *Enterococcus faecalis* is a major challenge due to its ability to penetrate dentinal tubules, survive harsh conditions, and form biofilms, leading to persistent apical periodontitis and root canal treatment failure (Siqueira & Rôças, 2014).

Calcium hydroxide has long been the standard intracanal medicament due to its high pH, broad antimicrobial spectrum, and ability to promote tissue healing and apexification in immature teeth (Mohammadi & Dummer, 2011). However, its effectiveness against resistant species like *E. faecalis* is inconsistent, and its long-term use can weaken dentinal walls and impair stem cell viability, limiting its use in regenerative endodontics (Sathorn & Parashos, 2007; Wigler et al., 2013).

Given these limitations, there is a growing need for alternative intracanal medicaments. *Nigella sativa* (black seed), especially its active compound thymoquinone, has shown promise as an antimicrobial, anti-inflammatory, and antioxidant agent (Kumar et al., 2020; Hosseinzadeh et al., 2014). Advances in nanobiotechnology can enhance its stability and dentinal penetration, offering improved effectiveness against resistant pathogens. Thymoquinone has been found to disrupt microbial membranes, inhibit ATP synthesis, and prevent biofilm formation (Forouzanfar et al., 2014).

While other botanical agents such as propolis, curcumin, and aloe vera have been explored, their limited solubility and inconsistent penetration restrict their use (Prabhakar et al., 2010). Nano-formulations of these compounds may improve their stability and deeper diffusion into dentinal tubules, potentially overcoming the limitations of calcium hydroxide (Al-Shwaimi, 2014).

These factors underscore the need for alternative medicaments with more consistent antimicrobial action and better biological compatibility.

## 2. LITERATURE REVIEW

Persistent microbial infections within the root canal system are a leading cause of endodontic treatment failure (Gijo et al., 2015). Microorganisms such as *Enterococcus faecalis* play a significant role in periapical and pulp diseases, and their persistence complicates endodontic procedures (Chandwani et al., 2022). The root canal system, deprived of immune system access, provides a conducive environment for microbial growth, making complete disinfection essential for successful treatment (Gijo et al., 2015). Chemomechanical preparation, combining mechanical instrumentation with chemical irrigants, is effective in reducing microbial populations, but it is insufficient to eliminate all contaminants. Therefore, intracanal medicaments are often used to enhance disinfection (Meire & van der Waal, 2022).

Calcium hydroxide is widely used as an intracanal medicament due to its ability to disrupt bacterial cell walls and prevent secondary infections (Momenjavid et al., 2022). However, its effectiveness against microbial biofilms, especially those formed by *E. faecalis*, is limited (Dako et al., 2020). This bacterium is highly resilient, capable of surviving in hostile conditions and evading conventional treatment methods (Seguel et al., 2020). As a result, alternative treatments, including the use of *Nigella sativa* (black cumin), are being explored.

*Nigella sativa*, particularly its active compound thymoquinone, has demonstrated significant antibacterial properties, including biofilm inhibition (Dajani Loyola-Chicago & Dajani, 2016). Studies suggest that *Nigella sativa* could serve as a promising alternative to calcium hydroxide for treating *E. faecalis* in root canals (Kumar et al., 2019). Further research is required to evaluate its safety, efficacy, and potential for clinical use in endodontics (Mashayekhi-Sardoo et al., 2020). Thus, *Nigella sativa* represents a potential solution for overcoming the limitations of current treatments, offering a natural, effective alternative for endodontic disinfection.

## 3. METHODOLOGY

### 3.1 Preparation of BHI Agar

30g of BHI agar was mixed with 1 L of purified water, heated until dissolved, and autoclaved at 121°C for 15 minutes. About 20 mL of molten agar was poured into Petri dishes, solidified, sealed, and stored at 4°C.

### 3.2 Preparation of Thymoquinone Stock Solution

100 mg of thymoquinone was dissolved in 10 mL of 10% DMSO (10 mg/mL). 1 mL aliquots were diluted with 9 mL distilled water to achieve a 1 mg/mL solution.

### 3.3 Preparation of Bacterial Stock Culture

*Enterococcus faecalis* was cultured overnight on BHI agar. A single colony was transferred to BHI broth and incubated at 37°C for 18 hours. A standardised suspension ( $1.0 \times 10^6$  cells/mL) was prepared by serial dilution and OD measurements.

### 3.4 Determination of MIC

MIC was determined using a 96-well plate with 100  $\mu$ L of BHI broth per well. 100  $\mu$ L of thymoquinone was added to the first well and two-fold serial dilutions were made. Control wells included blank, positive (*E. faecalis* + calcium hydroxide), and negative (*E. faecalis* only). Final concentrations tested were 100 to 0.2  $\mu$ L/mL. Plates were incubated at 37°C for 18 hours and growth was assessed by visual inspection and OD<sub>600nm</sub> measurements. All tests were performed in triplicate.

The percentage of bacterial growth inhibition was calculated using the following formula:

$$\% \text{ of Inhibition} = \left[ \frac{OD \text{ of Control} - OD \text{ of Test}}{OD \text{ of Control}} \right] \times 100\%$$

### 3.5 Determination of MBC

MBC was defined as the lowest thymoquinone concentration that killed 99.9% of *E. faecalis*. Samples from MIC wells with no growth were streaked onto BHI agar and incubated at 37°C for 18 hours. The lowest concentration with no bacterial growth was recorded as the MBC.

### 3.6 Adherence Inhibition Assay

Thymoquinone's effect on bacterial adherence was tested using smooth glass surfaces in test tubes. Bacterial suspensions in BHI broth with sub-MIC thymoquinone were incubated at 37°C for 18 hours.

- Positive control: BHI + *E. faecalis* (no thymoquinone)
- Negative control: BHI only

After incubation, non-adherent bacteria were removed by washing. Adherent bacteria were detached by sonication, and OD at 600 nm suspension was measured. All tests were done in triplicate.

### 3.7 Statistical Analysis

Data were analysed using SPSS. One-way ANOVA was used to assess differences between groups, and results were reported as mean  $\pm$  standard deviation. A significance level of  $P < 0.05$  was used. The Mann–Whitney U test compared MIC values between thymoquinone and calcium hydroxide to evaluate antimicrobial effectiveness.

## 4. RESULTS

Thymoquinone showed strong antibacterial effects against *Enterococcus faecalis*. The MIC of thymoquinone was 1.6  $\mu$ g/mL, much lower than calcium hydroxide (25  $\mu$ g/mL), showing that thymoquinone is more effective at lower concentrations.

Optical density (OD<sub>600</sub>) readings confirmed this result. Bacterial growth was highest at 0.2  $\mu$ g/mL (OD = 0.2288) and lowest at 100  $\mu$ g/mL (OD = 0.0039), showing that increasing thymoquinone concentration strongly reduces bacterial growth.

The percentage of bacterial inhibition increased with concentration. The highest inhibition was 99.80% at 100 µg/mL. At 1.6 µg/mL (MIC), inhibition was 92.21%, and at 3.12 µg/mL (MBC) it was 98.26%. Inhibition dropped sharply to 50.9% at 0.8 µg/mL.

Statistical tests supported by these findings.

- ANOVA showed a highly significant difference between concentrations ( $p < 0.001$ ).
- Tukey's test confirmed that 1.6 µg/mL caused a major drop in bacterial growth.
- The Mann–Whitney U test found a significant difference between thymoquinone and calcium hydroxide ( $p = 0.025$ ), proving that their antibacterial effects are not the same.

The MBC of thymoquinone was 3.12 µg/mL, while calcium hydroxide had an MBC of 50 µg/mL, showing that thymoquinone kills bacteria at much lower concentrations. All MBC results were consistent in three repeated tests.

Thymoquinone also reduced bacterial adherence to surfaces. The lowest adherence (lowest OD values) occurred at 1.6 µg/mL and 3.12 µg/mL. When the concentration decreased to 0.8, 0.4, and 0.2 µg/mL, adherence increased, meaning the anti-adherence effect weakened at lower doses.

Overall, thymoquinone showed strong, dose-dependent antibacterial and anti-adherence effects and performed better than calcium hydroxide in all major tests.

## 5. DISCUSSION

This study demonstrates that *Nigella sativa*, particularly its active component thymoquinone, shows significant potential as an alternative or adjunct to calcium hydroxide in endodontic treatments, especially for persistent *Enterococcus faecalis* infections. Thymoquinone's strong antibacterial and anti-adherence properties make it effective in counteracting biofilm formation, a key challenge in root canal therapy. Given the rise in antibiotic resistance among endodontic pathogens, natural alternatives like *Nigella sativa* may offer safer, less toxic, and more effective treatment options. The study also suggests that thymoquinone outperforms calcium hydroxide in antimicrobial efficacy and concentration, making it a promising candidate for improving treatment outcomes and addressing resistance issues in endodontics.

Future research should focus on in vivo studies to assess the safety and efficacy of *Nigella sativa* in clinical settings, explore its molecular mechanisms, and optimize its formulation to enhance its antibacterial properties. While the findings are promising, further investigations are needed to fully integrate *Nigella sativa* into clinical protocols for root canal therapy, offering a natural solution to antibiotic-resistant infections and improving dental health outcomes.

## 6. CONCLUSION

This study highlights the antibacterial efficacy of *Nigella sativa* aqueous extracts, particularly thymoquinone, against *Enterococcus faecalis*. Thymoquinone showed superior antimicrobial properties compared to calcium hydroxide, with lower Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values, as well as strong anti-adherence activity to prevent biofilm formation. Given the rise in antibiotic resistance, *Nigella sativa* offers a promising natural alternative in managing persistent root canal infections.

However, further research, including in vivo studies, is needed to validate its clinical application. Optimizing extraction methods, exploring molecular mechanisms, and assessing its safety and efficacy in clinical trials will be crucial for its integration into endodontic therapy. *Nigella sativa* could become a valuable adjunctive or alternative treatment, particularly for antibiotic-resistant infections like *E. faecalis*, improving patient outcomes in dental care.

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