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Original Article

Ex Vivo Evaluation of Benefect™ Essential Oil Disinfectant Against Endodontic Pathogens in Comparison with Sodium Hypochlorite and Chlorhexidine

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ABSTRACT

Natural antibacterial agents, including essential oils, are emerging as potential alternatives for endodontic disinfection due to their lower cytotoxicity compared with conventional irrigants such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX). Benefect™ is a proprietary essential oil formulation with broad-spectrum antibacterial properties. This study aimed to evaluate and compare the antibacterial effectiveness of Benefect™ with 6% NaOCl and 2% CHX against several endodontic pathogens. One hundred extracted human permanent single-canal teeth were decoronated, instrumented, and sterilized. The samples were subsequently inoculated with *Streptococcus mutans*, *Enterococcus faecalis*, *Actinomyces naeslundii*, or *Porphyromonas gingivalis* for 6–24 hours. The teeth were randomly assigned into four groups based on the irrigant used. Each sample was exposed to the respective irrigant for 12 minutes. Antibacterial efficacy was determined by quantifying viable bacteria relative to saline-treated controls. Statistical comparisons were performed using Student's t-test.
Treatment with NaOCl, CHX, or Benefect™ resulted in complete elimination of *S. mutans* (>99.9% reduction) compared with saline. Similarly, all tested irrigants achieved at least 99% bacterial reduction for *E. faecalis*, *A. naeslundii*, and *P. gingivalis*. No significant differences in antibacterial activity were observed among the three irrigants. Benefect™ botanical disinfectant demonstrates antibacterial efficacy comparable to 6% NaOCl and 2% CHX against *S. mutans*, *E. faecalis*, *A. naeslundii*, and *P. gingivalis*, suggesting its potential as a less toxic alternative for endodontic irrigation.

Keywords: Essential oil, Endodontic irrigation, Disinfection, Biofilm, Endodontic pathogens, *E. faecalis*

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Introduction

Microbial colonization is the principal factor driving endodontic infections [1]. These infections develop when the root canal system is exposed to the oral cavity, particularly under conditions of reduced local host immunity [2]. Microorganisms may gain access through carious lesions or traumatic damage to the coronal tooth structure. Without intervention, bacterial pathogens and their toxic by-products can migrate through the apical foramen and affect the surrounding periradicular tissues [3].

Endodontic infections are classified as primary, secondary, or persistent depending on the timing of microbial invasion into the pulp space [4]. Primary infections arise from initial colonization of necrotic pulp tissue. Secondary infections result from microorganisms introduced after clinical procedures, either accidentally during treatment or through coronal microleakage. Persistent infections occur when

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microorganisms from primary or secondary infections survive despite chemo-mechanical debridement, thriving in the nutrient-poor environment of treated canals. Clinically, distinguishing secondary from persistent infections is often challenging, and they are frequently grouped together [5].

Primary infections are typically dominated by Gram-negative strict anaerobes such as *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Tannerella*, and *Treponema* species [5]. Secondary and persistent infections, however, tend to involve fewer microbial taxa—mainly Gram-positive facultative anaerobes including *Streptococcus*, *Lactobacillus*, *Actinomyces*, *Enterococcus*, as well as *Candida albicans* [3, 6, 7].

Effective endodontic therapy aims to eliminate inflamed pulp tissue along with associated microorganisms. Mechanical instrumentation alone often fails to achieve complete disinfection, making irrigating solutions essential for reducing bacterial load throughout the root canal system [8]. An ideal irrigant should provide broad-spectrum antimicrobial activity, target both facultative and obligate anaerobes within biofilms, neutralize endotoxins, dissolve pulp remnants, avoid smear layer formation, and be biocompatible with periapical tissues [9].

Sodium hypochlorite (NaOCl) remains the most widely used irrigant due to its combined antimicrobial and tissue-dissolving properties [9, 10]. Its efficacy, especially against *E. faecalis*, is concentration-dependent, though high concentrations (5–9%) risk damage to the dentin matrix and cytotoxic effects if extruded beyond the apex [11, 12]. Chlorhexidine (CHX) is valued for its broad antimicrobial spectrum and substantivity [13], but it does not dissolve organic tissue, may be cytotoxic to periapical tissues, and is less effective against mature biofilms compared with early biofilms [14–16].

Due to these limitations, there is growing interest in natural compounds as potential endodontic irrigants [17]. Plant-derived agents often possess antimicrobial, anti-inflammatory, and antioxidant properties, making them attractive alternatives for root canal disinfection [18]. Benefect™, a novel essential oil–based formulation, has demonstrated broad-spectrum antimicrobial activity, including bactericidal, fungicidal, virucidal, and tuberculocidal effects, primarily by disrupting microbial cell membranes. Its composition includes thyme oil (0.23%), lemongrass oil (0.1–1.0%), biosurfactants, and ionized water. Importantly, Benefect™ is classified by the U.S. Environmental Protection Agency as having the lowest toxicity rating across all exposure routes, including inhalation, ingestion, and skin or eye contact [19].

This study investigates the antibacterial performance of Benefect™ in comparison with 6% NaOCl and 2% CHX against key endodontic pathogens: *Streptococcus mutans*, *Enterococcus faecalis*, *Actinomyces naeslundii*, and *Porphyromonas gingivalis*.

Materials and Methods

Ethical approval and sample selection

The study protocol was reviewed and granted an exemption by the University of Detroit Mercy Institutional Review Board (#23-24-17, 15 August 2023), in compliance with DHHS human subjects regulations. One hundred mature, single-rooted human teeth, extracted for orthodontic or periodontal reasons, were collected. Teeth exhibiting caries, fractures, root resorption, or calcified canals were excluded. All specimens were initially cleaned and stored in 3% sodium hypochlorite at room temperature. Radiographic assessment in two planes confirmed single canal anatomy for each tooth.

Tooth preparation

Crowns were removed at the cemento-enamel junction using a high-speed handpiece and #557 bur (Komet USA). The apical portion of each canal was prepared to ISO size #20 through sequential use of hand K-files (#8, #10, #15, and #20; MANI, Japan). During instrumentation, 5 mL of sterile saline was delivered via a 30-G side-vented needle (ProRinse, Dentsply-Maillefer) to maintain canal hydration and remove debris. Composite resin was used to seal the apices, and the outer root surfaces were coated with nail polish to prevent leakage and external contamination. Prepared teeth were stored in sterile saline until further use.

Sterilization

Prepared specimens were sterilized by autoclaving at 121 °C for 15 minutes. Following sterilization, teeth were stored in sterile 1× phosphate-buffered saline (PBS; Corning) for a minimum of 48 hours to maintain aseptic conditions.

Bacterial cultures and inoculation

Four bacterial species were selected to represent common endodontic pathogens: *Enterococcus faecalis* (ATCC 29212), *Actinomyces naeslundii* (ATCC 12104), *Streptococcus mutans* (ATCC 25175), and *Porphyromonas gingivalis* (ATCC 33277). Overnight cultures of *S. mutans*, *E. faecalis*, and *A. naeslundii* were grown in brain heart infusion (BHI) at 37 °C, with 5% CO_2 for *S. mutans* and *A. naeslundii*. *P. gingivalis* was grown under anaerobic conditions in brucella broth supplemented with vitamin K (0.5 µg/mL) and

hemin (5 µg/mL). Cultures were centrifuged at 4500 rpm for 10 minutes, washed with PBS, and resuspended to OD_{600} = 1.0 in artificial saliva containing 0.5% sucrose (except *P. gingivalis*, which was resuspended in supplemented brucella broth). Approximately 1 × 10^6 CFU of each bacterial suspension was introduced into the prepared canals. Teeth were incubated to allow bacterial attachment: *S. mutans* and *A. naeslundii* at 37 °C with 5% CO_2, *E. faecalis* at 37 °C aerobically, and *P. gingivalis* under anaerobic conditions for 4–6 hours.

Instrumentation and irrigation protocol

Canals were prepared using Protaper Ultimate F1, F2, and F3 rotary files (Dentsply Sirona). During instrumentation, each canal received irrigation with the assigned solution—Benefect™ (Sensible Life Products, Canada), 6% NaOCl (Pure Bright, Canada), 2% CHX (Vista Apex, USA), or sterile saline—applied in 3 mL aliquots every 4 minutes, totaling 12 minutes of contact time per canal [17]. After instrumentation, canals were flushed with 10 mL sterile saline over 2 minutes to remove any residual irrigant.

Figure 1. Overview of Experimental Procedure

The study setup included a ProMark endodontic motor (Dentsply Tulsa Dental, Johnson City, TN, USA) and Protaper Ultimate rotary files (F1, F2, F3; Dentsply Sirona, Charlotte, NC, USA). Irrigation solutions—6% NaOCl, 2% CHX, sterile saline, and Benefect™—were placed in separate sterile containers. All procedures were carried out within a disinfected chamber to prevent external contamination during instrumentation and irrigation of inoculated teeth.

Bacterial sampling and quantification

Following canal instrumentation, sterile paper points were inserted into each root canal for one minute to absorb residual bacteria. Points were then transferred aseptically into 0.5 mL of sterile PBS and vortexed for 10 seconds to detach bacterial cells. Serial ten-fold dilutions (50 µL) were plated on Brain Heart Infusion agar or Brucella Blood Agar supplemented with 5% sheep blood, vitamin K (0.5 µg/mL), and hemin (5 µg/mL). Plates were incubated under appropriate conditions for 2–7 days to allow colony formation. Antibacterial effectiveness was calculated relative to saline-treated controls. To confirm successful colonization, some teeth were sampled without irrigation or instrumentation.

Liquid culture assay for P. gingivalis

P. gingivalis cultures were prepared two days prior in 10 mL brucella broth with vitamin K (0.5 µg/mL) and hemin (5 µg/mL) under anaerobic conditions. Cultures were diluted 1:10 in fresh medium one day before testing. On the day of the assay, bacterial suspensions were adjusted to OD_{600} = 1.0 in sterile PBS. Aliquots of each test solution (0.9% saline, 6% NaOCl, 2% CHX, Benefect™; 450 µL) were mixed with 50 µL bacterial suspension and incubated at room temperature for 12 minutes. Samples were centrifuged at 7000 rpm for 5 minutes, the supernatant removed, and the pellet resuspended in 450 µL PBS. Serial dilutions were prepared, plated on Brucella Blood Agar, and incubated anaerobically for nine days to assess colony formation.

Results and Discussion

The antimicrobial activity of Benefect™ was compared with NaOCl and CHX in teeth colonized with different endodontic pathogens. Bacterial load in saline-treated canals was set as 100%, and reductions following treatment were quantified. Benefect™ completely eliminated *S. mutans* colonies (>99.9% reduction), similar to NaOCl and CHX **(Figure 2a)**. For *E. faecalis* and *A. naeslundii*, Benefect™ achieved approximately 99% reduction, with no statistically significant difference compared to the other irrigants **(Figures 2b and 2c)**.

For *P. gingivalis*, reductions in colony counts after irrigation with any of the three antimicrobials were not statistically significant compared with saline, due to uneven colonization in some specimens **(Figure 2d)**. To directly measure susceptibility, a liquid killing assay was performed. In this setup, all three treatments—Benefect™, NaOCl, and CHX—achieved >99.9999% bacterial killing, confirming high efficacy outside the tooth environment **(Figure 3)**. Experiments were conducted in 2–3 independent trials with duplicates (n = 4–6), and data were analyzed using Student's t-test.

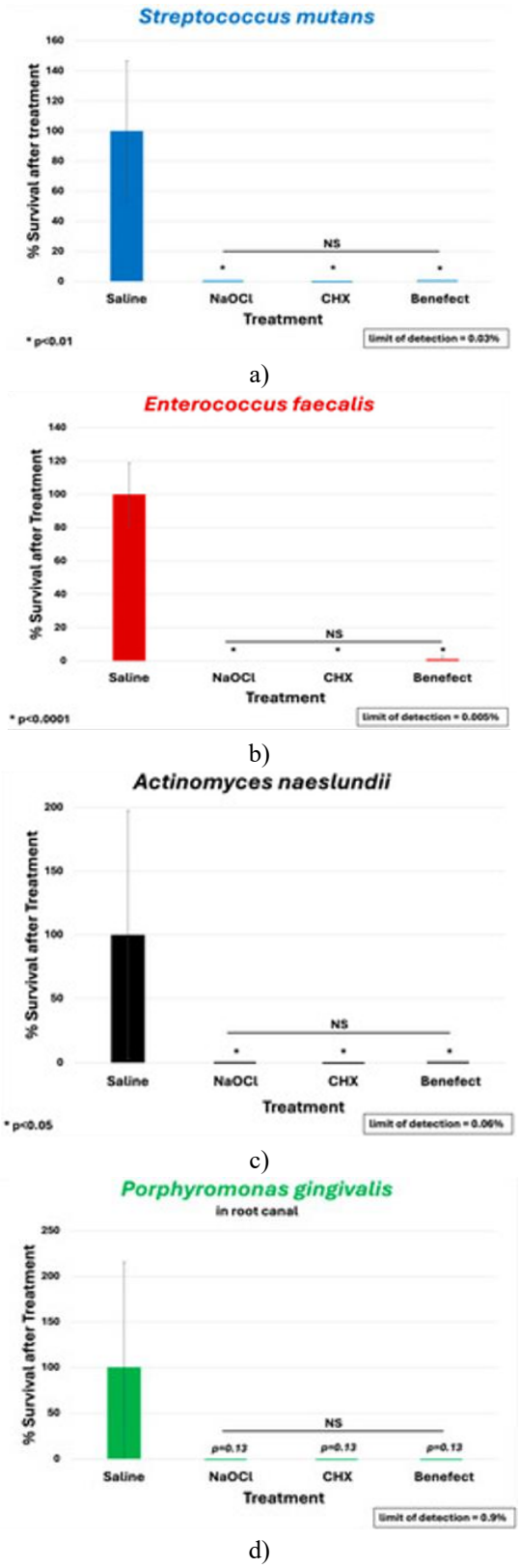

Figure 2. Comparative root canal disinfection by Benefect™, NaOCl, and CHX. Benefect™ achieved complete elimination of S. mutans colonies (>99.9% reduction) relative to saline, showing similar efficacy to both NaOCl and CHX (A). NS indicates no statistically significant differences among groups. Benefect™ also produced approximately 99% reduction in E. faecalis (B) and A. naeslundii (C), comparable to the other irrigants. Treatment of P. gingivalis with Benefect™ resulted in 99% bacterial reduction (D); however, due to variable colonization in some specimens, this reduction was not statistically significant compared with saline (p = 0.13).

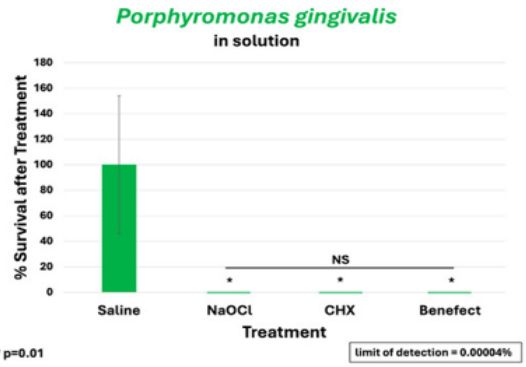

Figure 3. Benefect™ is as effective as NaOCl and CHX in eliminating P. gingivalis in liquid-killing assay. Benefect™ and other treatments all resulted in >99.9999% killing efficacy with no difference among all treatment groups.

This study represents the first evaluation of Benefect™ as a potential endodontic irrigant, specifically assessing a combined formulation of thyme and lemongrass essential oils. Our results demonstrate that Benefect™ achieves antibacterial activity in the root canal comparable to conventional irrigants such as NaOCl and CHX.

Previous research has reported antimicrobial properties of the individual oils contained in Benefect™. For instance, in vitro comparisons of endodontic sealers—zinc oxide with eugenol versus zinc oxide with thyme oil—showed that the thyme-containing formulation had stronger activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa* [20]. In vivo studies have also confirmed that thyme-based irrigants can significantly reduce *E. faecalis* populations [21]. Oregano oil has similarly demonstrated potent antibacterial activity, with certain concentrations matching or exceeding the efficacy of 5.25% NaOCl against *E. faecalis* [22, 23].

Lemongrass oil has been shown to inhibit both *E. faecalis* and *Candida albicans*, and in some studies, lemongrass extracts outperformed 2.5% NaOCl in suppressing *E. faecalis* growth [24, 25]. Other investigations examining thyme, oregano, and lemongrass oils in instrumented and non-instrumented canals confirmed that these essential oils can significantly reduce bacterial loads, with thyme and

oregano achieving near-complete eradication, while lemongrass reduced bacteria by over 75% [26].

Our findings suggest that the combination of thyme and lemongrass oils, when used alongside mechanical instrumentation, provides an antibacterial effect comparable to NaOCl and CHX. This aligns with prior studies, such as Nagy-Bota *et al*., 2021 [26], though our study is unique in evaluating the oils as a combined formulation. Beyond *E. faecalis*, we also investigated the effect of Benefect™ on three additional endodontic pathogens, supporting evidence that natural antimicrobial agents can be broadly effective. Similar observations were reported by Pedrinha *et al*., 2025 [27], who found that propolis and copaiba resin exhibited comparable activity to conventional agents against dual-species biofilms.

Future research should focus on assessing Benefect™ against polymicrobial biofilms typical of secondary or persistent endodontic infections, as well as evaluating its cytotoxicity toward periapical tissues. Limitations of this study include the exclusive use of single-rooted teeth; complex canal anatomies in multirooted teeth, including isthmuses, fins, and accessory canals, may influence irrigant efficacy. Additionally, microbial sampling with paper points, though standardized, may introduce variability in bacterial recovery, and while contamination was not observed in our study, it remains a potential concern. Lastly, monospecies biofilms were employed, whereas clinical endodontic infections are polymicrobial. Developing stable in vitro polymicrobial biofilms remains challenging, but such models could be used to further evaluate Benefect™ in future studies.

Conclusion

Benefect™ demonstrates antibacterial activity in root canals comparable to 6% NaOCl and 2% CHX. These results suggest that Benefect™ may serve as a natural, effective alternative to conventional chemical irrigants in endodontic treatment.

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Conflict of Interest: None

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