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## Evaluating the Antibacterial Effectiveness of a Novel Intra-Canal Medicament Against *Enterococcus faecalis* Under Hyperthermic Conditions: An In Vitro Study

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### ABSTRACT

This study aims to compare the efficacy of three types of intracanal medicaments in eliminating *Enterococcus faecalis* in cases of periapical abscess with systemic fever. The persistence of resistant microorganisms, such as *Enterococcus faecalis*, contributes to the failure of endodontic treatment, a very common problem in clinical practice. This study is particularly relevant under febrile conditions. The research addresses the challenge of eradicating resistant bacterial strains that cause endodontic infections, a significant factor in the failure of various intracanal medicaments. The study highlights the importance of Hansedent Endomix, which was developed by Dr. Aziz Abdullah and patented under number 6075 on December 20, 2016. The study evaluates the antibacterial efficacy of Hansedent Endomix against *Enterococcus faecalis* and compares it with Metronidazole and Ledermix in febrile conditions. The findings revealed that Hansedent Endomix demonstrated significantly higher antibacterial activity, as indicated by the inhibition zone diameter after 24 and 48 hours, in a sample of three plates against *Enterococcus faecalis*. At a 5% significance level, Hansedent Endomix showed superior results compared to Metronidazole and Ledermix under febrile conditions. This study provides valuable insights into the superior efficacy of Hansedent Endomix and its potential application in resistant endodontic infections.

**Keywords** Hansend Endomix, *Enterococcus faecalis*, Intra canal medicaments, Antibacterial efficacy, Endodontic infections



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### Introduction

The success of endodontic treatment relies on the complete elimination of microorganisms through mechanical preparation, irrigation, microbial control, and thorough root canal obturation. Microorganisms and their byproducts contribute to pulp necrosis and periapical lesions. However, these microorganisms may survive endodontic procedures due to anatomical complexities that limit the access of instruments

and irrigation solutions [1]. The primary goal of endodontic treatment is to remove microbial infections from the root canal system. Microorganisms can adhere to one another within the root canal space, forming biofilms that attach to dentinal walls, thereby obstructing effective disinfection. Cleaning and disinfection can be enhanced through mechanical and chemical means, such as preparation and irrigation with endodontic solutions [2]. With the increasing prevalence of odontogenic infections, treatment methods have gained significant importance. The selection of appropriate disinfecting medicaments is critical, especially given the challenges posed by complex canal anatomy. Additionally, improper cleaning and disinfection of the root canal space exacerbate the problem, with residual microorganisms being a leading cause of endodontic treatment failure [3]. Chemical auxiliaries are essential for achieving successful cleaning and disinfection of root canals [4]. These agents can be employed Intracanal medicaments are considered a fundamental step in killing bacteria within root canals [5]. The use of intracanal medicaments provides clinicians with the opportunity to verify the effectiveness of endodontic procedures. If short-term results are satisfactory, the clinician can proceed with completing the treatment [6]. *Enterococcus faecalis* is a facultatively anaerobic, gram-positive bacterium and is one of the most resistant microorganisms in the root canal system. Although it constitutes a small percentage of the root canal flora in primary endodontic infections, environmental changes can favor *E. faecalis*, leading to persistent infection. Some resistance factors of this bacterium include the ability to penetrate deeply into dentin, tolerate high pH, and survive even in nutrient-deprived conditions [7]. This study aims to compare the effectiveness of three types of intracanal medicaments in eliminating *Enterococcus faecalis* under hyperthermic conditions.

Bacterial infection associated with endodontic treatment failure is a common challenge in clinical practice, with *Enterococcus faecalis* being one of the most prominent causative agents due to its relative resistance to elimination. In hyperthermic conditions, the ability of these bacteria to cause infection may increase due to the patient's weakened immunity. Despite the existence of various currently used medicaments, their effectiveness in conditions similar to fever remains not precisely determined. Hence, there is a need to find a new effective medicament against bacterial presence at elevated temperatures. This study highlights the effectiveness of Hanesdent Endomix medicament under conditions close to clinical reality and provides a comparative evaluation between medical materials used in clinical practice, taking into account body temperature when studying antibacterial effectiveness, an aspect that remains rarely addressed in previous studies.

The primary objective of this study was to evaluate the antibacterial effectiveness of Hanesdent Endomix medicament against *Enterococcus faecalis* at a temperature simulating hyperthermic conditions. Secondary objectives included comparing the effectiveness of Hanesdent Endomix medicament with other traditionally used medicaments, evaluating the continuation of effectiveness over periods of 24 and 48 hours, and analyzing the effect of elevated temperature on the effectiveness of different medicaments.

## Methods

### Study Design and Setting

This laboratory study was conducted in the Microbiology Laboratory of the Department of Plant Life at the Higher Institute for Environmental Sciences, Faculty of Science, Tishreen University in 2024.

## **Materials**

Three intracanal medicaments were used, mixed with distilled water as shown in Table 1:

1. Hansedent Endomix, a novel herbal-based intracanal medicament with additional pharmaceutical components, developed by Dr. Aziz Abdullah and patented under number 6075 dated 12/20/2016. The medicament consists of an aqueous-consistency vehicle loaded with herbal extracts including Tea Tree Oil (3-5%), Chamomile flower (0.1-0.2%), Hydrocortisone (0.2-0.3%), and Nystatin (2%).
2. Ledermix from RIEMSER PHARMA GMBH, GERMANY, containing Demeclocycline hydrochloride (3.2%) and Triamcinolone acetonide (1%) in a polyethylene glycol base.
3. Metapaste from META BIOMED CO., KOREA, containing Calcium hydroxide (38%) and Barium sulfate (30%) in a methylcellulose base.

The bacteria studied were facultatively anaerobic, gram-positive *Enterococcus faecalis* bacteria, obtained isolated and pure, grown in nutrient broth medium at 37°C and a 24-hour incubation period, then cultured on nutrient agar medium under the same conditions. The sample was renewed weekly to ensure obtaining a pure strain and preserved in the refrigerator at 4°C.

## **Methodology**

The Agar Disk Diffusion Method was used to evaluate the antimicrobial effectiveness of the medicaments. The sample consisted of 12 antibiotic discs divided into four groups distributed across three Petri dishes as illustrated in Figure 1:

- Group One: Hansedent Endomix medicament group (three discs)
- Group Two: Ledermix medicament group (three discs)
- Group Three: Metapaste medicament group (three discs)
- Group Four: Distilled water as a control group (three discs)

Mueller-Hinton agar was prepared by dissolving 38g in 1L of distilled water on a magnetic heater until dissolution, and sterilized in an autoclave at a temperature of 121°C for 20 minutes, then left to reach a temperature of 50°C, then poured into Petri dishes with a diameter of 90mm, and approximately 15mL was poured into each dish and left to cool.

A bacterial suspension of *E. faecalis* was prepared and adjusted to 0.5 McFarland standard. The medicaments were prepared at a 1:1 dilution with distilled water and applied to paper discs. The discs were placed on the agar plates inoculated with *E. faecalis* and incubated at 38-39°C to simulate hyperthermic conditions. Inhibition zones were measured after 24 and 48 hours, with results documented in Figure 2 and Figure 3 for the 24-hour and 48-hour periods, respectively.

### Statistical Analysis

To achieve the research objectives, the Statistical Package for Social Sciences (SPSS V20) was used to conduct the analysis with a significance level of 5%, corresponding to a confidence level of 95%, employing a comprehensive approach that included the Kolmogorov-Smirnov normality test to verify data distribution, followed by paired sample t-test for comparing measurements at different time points, one-way ANOVA for comparing between groups, Bonferroni test for post-hoc analysis, and calculation of absolute and relative frequencies for descriptive statistics.

### Results

The Kolmogorov-Smirnov normality test showed that the data followed a normal distribution ( $p$ -value $>0.05$ ) for all research data, therefore parametric tests were used. As shown in Table 2, the highest mean inhibition zone diameter was observed with Hansedent Endomix medicament (20.00 mm), while the other medicaments had the same diameter of 6.00 mm (disc diameter), indicating no inhibition zone. The mean values of the inhibition zone diameter remained stable between the two measurement periods (24 and 48 hours).

One-way ANOVA showed statistically significant differences between the four samples during both study periods ( $p<0.05$ ). Bonferroni test revealed that the significant difference was between Hansedent Endomix and the other medicaments, with the mean inhibition zone diameter of Hansedent Endomix being more than three times that of the other medicaments. The results of the sensitivity and resistance test, as presented in Table 3, showed that *E. faecalis* was sensitive to Hansedent Endomix medicament at 38-39°C, while it was resistant to both Ledermix and Metapaste medicaments at the same temperature.

### Discussion

*Enterococcus faecalis* is one of the most resistant microorganisms associated with failed endodontic treatment and endodontic infection. These bacteria possess an exceptional ability to remain viable within root canals even after completing the traditional preparation and disinfection process, as they can adapt to environments with limited nutritional resources and form biofilms that contribute to enhancing their resistance to chemical agents and antibiotics [8, 9]. The results of this study showed that Hansedent Endomix medicament demonstrated clear antimicrobial effectiveness against *E. faecalis* bacteria with recording clear bacterial inhibition zones as evident in Figure 2 and Figure 3. This effectiveness may be attributed to the multiplicity of biological effect mechanisms and their synergy as a result of the diversity of compounds in this medicament, with the stability of the medicament's composition at relatively high temperatures.

The antimicrobial properties of Hansedent Endomix can be attributed to its components as detailed in Table 1. Alpha-Bisabolol ( $\alpha$ -Bisabolol), a natural compound extracted from the essential oils of plants such as *Matricaria chamomilla* (German chamomile), has been shown to inhibit the growth of a wide range of microorganisms through a mechanism that includes causing changes in cell membrane permeability [10]. Tea Tree Oil contains terpinene-4-ol compound, which has strong antimicrobial properties, and studies have proven its effectiveness against a wide range of bacteria, including *E. faecalis* [11]. Ledermix medicament showed an absence of antimicrobial effectiveness against *E. faecalis* bacteria, which may be attributed to the effect of the active components in this medicament by the rise in temperature, leading

to loss of effectiveness. This finding agrees with the study of [13] that Ledermix medicament did not have a clear effect on *Enterococcus faecalis* bacteria.

Metapaste medicament showed zero effectiveness against *E. faecalis* bacteria despite containing calcium hydroxide, as shown in Table 3. This may be attributed to the fact that calcium hydroxide may need a relatively longer time to achieve the full effect [12]. Additionally, the nature of the medicament preparation base (polypropylene glycol) may affect the material's ability to spread within the solid medium. The results of this study agreed with the study of [14] regarding the clear effectiveness of Hansedent Endomix medicament against *Enterococcus faecalis*, and the absence of effectiveness of Metapaste medicament against *Enterococcus faecalis* bacteria. The results also agreed with the study of [15] that calcium hydroxide did not eliminate *Enterococcus faecalis* bacteria.

### **Conclusions**

Through this research and in light of the results obtained, we conclude the following:

1. Hansedent Endomix medicament showed clear antimicrobial effectiveness against *Enterococcus faecalis* bacteria, continuing after 24 hours to 48 hours under hyperthermic conditions (temperature 38-39°C).
2. Ledermix medicament did not show any antimicrobial effectiveness against *Enterococcus faecalis* bacteria after 24 hours and 48 hours under hyperthermic conditions (temperature 38-39°C).
3. Metapaste medicament did not show any antimicrobial effectiveness against *Enterococcus faecalis* bacteria after 24 hours and 48 hours under hyperthermic conditions (temperature 38-39°C).

Based on these findings, we recommend the use of Hansedent Endomix medicament as an effective medicament inside the root canal in cases infected with bacterial infection and expected to contain *Enterococcus faecalis* bacteria, in cases of endodontic treatment failure that do not respond to traditional medicaments, and in cases where there is inflammation or hyperthermic condition.

Future research should include clinical studies on Hansedent Endomix medicament to evaluate its antimicrobial effectiveness against *Enterococcus faecalis* bacteria in cases of hyperthermic conditions, studies on extracted human tooth roots, investigations of its effectiveness in different conditions inside the infected root canal, and studies against other types of microorganisms causing endodontic treatment failure.

### **Data Availability**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Tables and Figures

**Table 1: Composition of medicaments used in the research**

Medicament	Composition
Hansedent Endomix	Tea Tree Oil (3-5%), Chamomile extract (0.1-0.2%), Hydrocortisone (0.2-0.3%), Nystatin (2%) in an aqueous vehicle
Ledermix	Demeclocycline hydrochloride (3.2%), Triamcinolone acetonide (1%) in a polyethylene glycol base
Metapaste	Calcium hydroxide (38%), Barium sulfate (30%) in a methylcellulose base

**Table 2: Descriptive statistics for the inhibition zone diameters**

Medicament	Time	Mean (mm)	Standard Deviation	Minimum	Maximum
Hansedent Endomix	24 hours	20.00	1.00	19.00	21.00
	48 hours	20.00	1.00	19.00	21.00
Ledermix	24 hours	6.00	0.00	6.00	6.00
	48 hours	6.00	0.00	6.00	6.00
Metapaste	24 hours	6.00	0.00	6.00	6.00
	48 hours	6.00	0.00	6.00	6.00
Distilled water (control)	24 hours	6.00	0.00	6.00	6.00
	48 hours	6.00	0.00	6.00	6.00

**Table 3: Results of the sensitivity and resistance test for the three medicaments against *E. faecalis***

Medicament	Result	Inhibition Zone Diameter (mm)
Hansedent Endomix	Sensitive (S)	20
Ledermix	Resistant (R)	6
Metapaste	Resistant (R)	6
Distilled water (control)	Resistant (R)	6

Figure 1: Medicaments used in the research (A) Hansedent Endomix, (B) Ledermix, (C) Metapaste

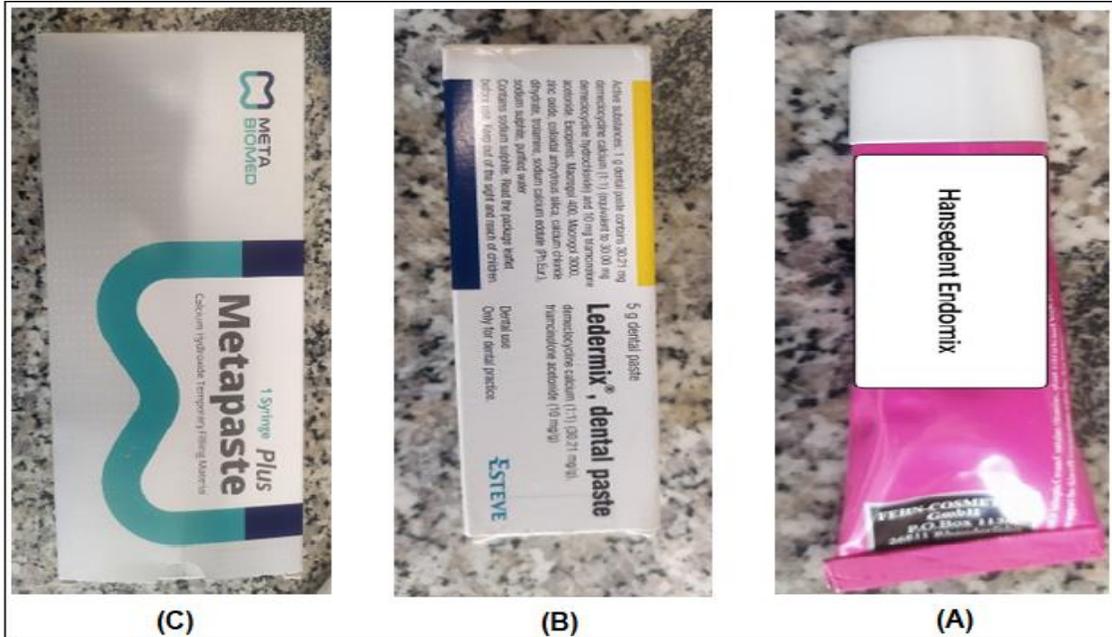
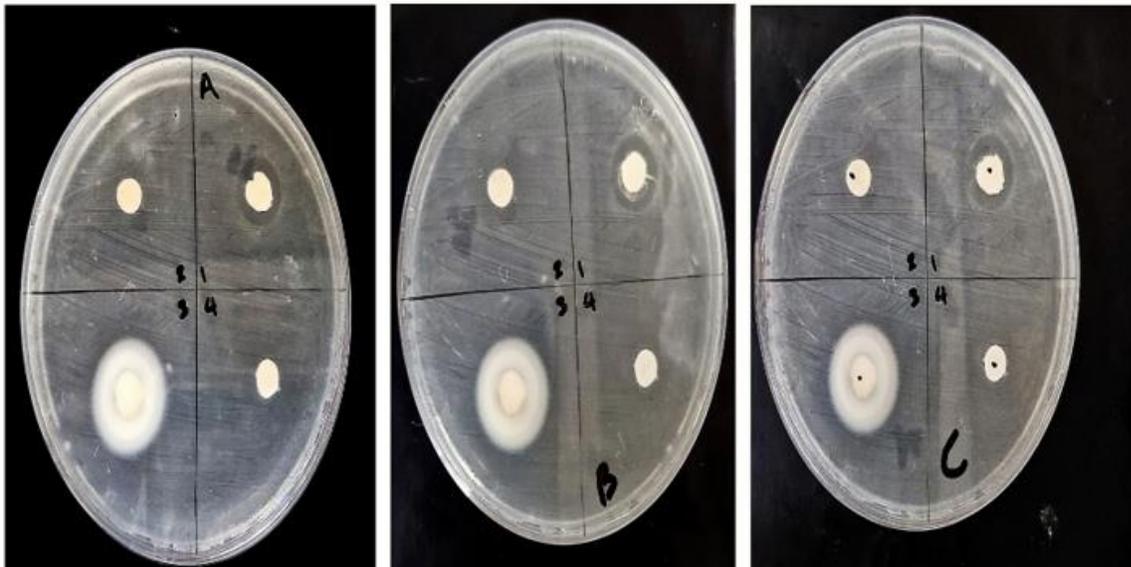
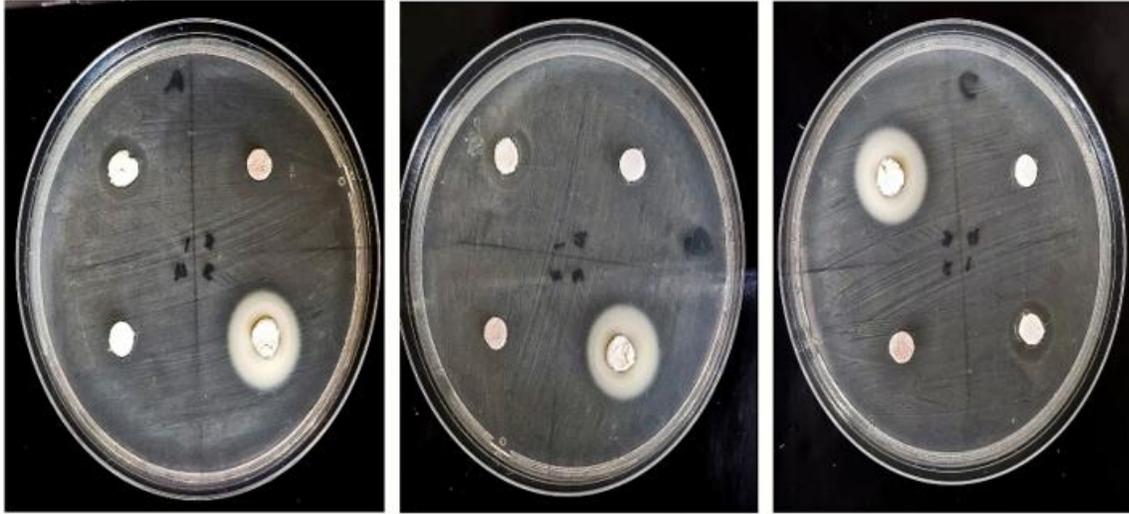


Figure 2: Results after 24 hours of incubation Petri dishes showing inhibition zones around discs impregnated with different medicaments



**Figure 3: Results after 48 hours of incubation** Petri dishes showing inhibition zones around discs impregnated with different medicaments



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