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Comparative Evaluation of Antimicrobial Efficacy of Fennel Seed Extract and 2 % Chlorhexidine as Root Canal Irrigants Against *E.Faecalis, S.Aureus* And *C.Albicans*

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KEYWORDS

Comparative Evaluation, Antimicrobial Efficacy

ABSTRACT:

Introduction: The primary cause of pulpal and periapical infection is the colonization of microorganisms which include gram positive, negative and anaerobic organisms. The goal of root canal treatment includes thorough disinfect the root canal system and preventing further infection. Three main steps of root canal treatment includes access cavity preparation, chemo-mechanical debridement and obturation. The most commonly used intra canal irrigants include NaOCl,CHX and EDTA. However these conventional endodontic irrigants have many adverse effects like cytotoxicity, irritation and cross reactions with different irrigant. Therefore, there is a need for a better or less toxic irrigant which can replace the conventional ones. Studies have proven that herbal irrigants of plant origin or its derivatives have good antibacterial activity and has a potential to be used as a root canal irrigant. Fennel (*Foeniculum vulgare Mill*) is an annual herbaceous plant belongs to the family Apiaceae. According to literature and previous studies conducted alcoholic and aqueous extracts of fennel seeds has antimicrobial action against different dental pathogens.

Objectives:.

To prepare fennel seed extract as a root canal irrigant and evaluate its antimicrobial efficacy against *E.faecalis*, *S.aureus*, and *C.albicans* in comparison with 2% chlorhexidine

Methods: 90 anonymized single rooted premolars extracted for orthodontic purpose were used for this study. *E.faecalis, S.aureus and C.albicans* were inoculated on separate tooth specimens and were incubated for 21 days for biofilm formation. The inoculated specimens where divided into 3 groups – group 1 (specimens inoculated with *E.faecalis*), group 2 (specimens inoculated with *S.aureus*) and group 3 (specimens inoclulated with *C.albicans*) with 30 samples each . The root canals was passively irrigated with 5ml irrigant according to the allocated groups. It was repeated for 4 times after which the dentinal shavings were collected from the root canal with H files. The H files was then placed in eppendroff tube and stirred for 30 seconds. 0.1 ml aliquot of each content was seeded and duplicated into dishes containing NB for *E.faecalis and S.aureus* and SDB for *C.albicans*. the characteristic bacterial and fungal colonies were counted and assessed.

Results: Significant differences were observed among the three groups for *S. aureus*, *E. faecalis*, and *C. albicans* ($p < 0.001$).

Conclusions: The study demonstrated statistically significant differences in CFU (*S. aureus*, *E. faecalis*, and *C. albicans*) among the three groups. Fennel seed extract has antimicrobial action against all the tested microbes but not as comparable to that of CHX.

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Introduction

Endodontics is the branch of dentistry concerned with diagnosis, prevention, and treatment of diseases of the dental pulp and periapical tissues. Root canal infections may be primary, arising from caries or trauma, or secondary/persistent,following unsuccessful treatment. Microbial invasion of the pulp and root canals can lead to acute or chronic periapical inflammation depending on virulence and extent of infection[1,2].

Endodontic therapy in primary teeth maintains them until natural exfoliation, while in permanent teeth it preserves structure and function[1,2]. The ultimate goal is to eradicate infection, restore function, and prevent reinfection[3]. According to Schilder, successful treatment requires "cleaning and shaping" of canals, which facilitates three-dimensional sealing[4,5]. Mechanical instrumentation alone cannot reach complex canal anatomy, so chemical irrigants are essential[6,7].

The most commonly used irrigants are sodium hypochlorite (NaOCl), chlorhexidine (CHX), and EDTA. NaOCl has potent antimicrobial and tissue-dissolving properties but is cytotoxic and irritating at higher concentrations[8,9]. CHX provides broad-spectrum, long-lasting antimicrobial action but lacks tissue-dissolving capacity and may form toxic precipitates with NaOCl [10,11,12]. EDTA removes the smear layer but reduces NaOCl activity and weakens dentin[9,13]. Adverse effects of these irrigants highlight the need for safer, biocompatible alternatives[14,15].

Growing concerns over cytotoxicity and microbial resistance have shifted interest toward herbal irrigants, which offer antimicrobial, anti-inflammatory, and antioxidant properties[16]. One such agent is fennel (Foeniculum vulgare Mill.), a traditional medicinal plant of the Apiaceae family. Historically used for tooth pain in ancient Egypt[17], fennel exhibits digestive, anticancer, anti-inflammatory, and antioxidant benefits[18,19]. Its essential oils and extracts show antimicrobial effects against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas*, *Klebsiella*, and oral pathogens including *Streptococcus mutans*[20,21]. Despite this, its potential as a root canal irrigant remains unexplored.

Objectives

Among endodontic pathogens, *Enterococcus faecalis* accounts for 24–77% of persistent infections[22]. Its ability to penetrate dentinal tubules and resist medicaments makes it a frequent cause of retreatment failure[22]. *Staphylococcus aureus* and *Candida albicans* are also associated with failed cases due to their dentinophilic and opportunistic nature[22].

Thus, this study evaluated and compared the antimicrobial efficacy of fennel seed extract and 2% chlorhexidine as root canal irrigants against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*.

Methods

This in vitro experimental study was conducted at Yenepoya Dental College under assistance of Yenepoya Research Centre, Mangalore, using 90 single-rooted intact permanent teeth collected from the Department of Oral and Maxillofacial Surgery and private clinics; a total of 90 samples (30 in each group)were included. Ethical clearance was obtained before starting the study [protocol numberYEC2/2023/040].

Preparation of fennel seed extract

1kg of fennel seeds were washed and dried followed by pulverization. Aqueous extract was obtained using refluxation method. 100g of powdered fennel seeds were mixed with 700ml of distilled water in a round bottom flask. It was fluxed for about 5 hours at 100˚C. The liquid extracts were filtered using whatman no:1 filter paper and concentrated using an electric water bath, dried and kept in air tight container . The extract was stored at 4 ˚C before the use.100g of the extract was added to 1000ml of distilled water and was allowed to dissolve. After filtration, the extract was autoclaved for 15min at 121 ˚C under 15lbs pressure.

Preparation of Tooth samples

A total of 90 anonymized, extracted human permanent single-rooted teeth indicated for extraction due to orthodontic reasons were used in this study. All teeth were inspected for cracks, resorption, or previous root canal treatment and any such teeth were excluded.

Initial Cleaning and Storage:The external surfaces of the teeth were cleaned using a scaler to remove any calculus and residual periodontal tissues, if present.The cleaned teeth were stored in normal saline at room temperature until further use to prevent dehydration.

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An endodontic access cavity was prepared using an endo-access bur under high-speed handpiece with water coolant.Canal orifices were enlarged using orifice openers/orifice enlargers.Working length was determined using the tactile sensation method by inserting a file until resistance was felt at the apex.Biomechanical preparation of the canal was carried out using the step-back technique up to a size #40 K-file, with intermittent irrigation using normal saline throughout the procedure.

Specimen Standardization: The teeth were decoronated at the level of the cementoenamel junction (CEJ) using a diamond-coated disk to standardize the root length.The apical foramen was sealed using Ionoseal (a light-cure glass ionomer cement) to prevent microbial leakage through the apex.The external surfaces of the root were coated with nail varnish, leaving only the cervical opening (access cavity) exposed to ensure a confined area for microbial testing.

Each specimen was placed in a glass beaker, covered with cotton and aluminum foil and were sterilized using autoclaving at 121°C for 20 minutes.After autoclaving, the specimens were stored in a hot air oven at 50°C until further use, maintaining a dry and sterile environment.

Preparation of Microbial Inoculum

Microorganisms Used:*Enterococcus faecalis*; *Staphylococcus aureus*; *Candida albicans*

2–3 colonies of each microorganism were collected from their respective mother plates.E. faecalis and S. aureus were inoculated into Nutrient Broth.C. albicans was inoculated into Sabouraud Dextrose Broth. All cultures were incubated overnight at 37°C in a rotating incubator until reaching an optical density equivalent to 0.5 McFarland standard.

30 sterile tooth specimens were used for each microorganism.1 mL of the standardized microbial suspension was added to each test tube containing a tooth specimen.Tubes were sealed and incubated at 37°C in a static incubator for 21 days.Microbial contamination was confirmed by the presence of turbidity.

After the contamination period, specimens were randomly divided into three groups (n = 10 per group)

for treatment: Fennel Seed Extract,2% Chlorhexidine (CHX),Normal Saline (NS)

Each infected canal was passively irrigated with 5 mL of the assigned irrigant using a 24-gauge needle and syringe. The procedure was repeated four times or more to ensure adequate exposure.

Microbial assessment

Microbial samples were collected immediately post irrigation. #35 H file was used to scrap the dentinal shavings from the canal walls of the treated specimens. The H-file was then placed in the eppendroff tube and stirred for 30seconds. A 0.1 mL aliquot of each content was seeded and duplicated into dishes containing Nutrient Broth for *E.faecalis* and *S.aureus* , and Sabouraud Dextrose Broth for *C.albicans*. Characteristic bacterial colonies were counted and assessed.

Results

The antimicrobial efficacy of fennel seed extract, 2% chlorhexidine, and normal saline was evaluated against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans* by measuring the colony-forming units (CFU) after treatment

For *E. faecalis*, the CFU counts after treatment with fennel seed extract ranged from 201 to 242, indicating moderate antimicrobial activity. In contrast, 2% chlorhexidine showed almost complete inhibition of microbial growth, with CFU values ranging from 0 to 6, mostly recording zero CFU. Normal saline exhibited the least antimicrobial effect, with high CFU values ranging from 940 to 998, indicating minimal inhibition of *E. faecalis* values ranging

In the case of *S. aureus*, the fennel seed extract showed CFU counts between 228 and 246, indicating antimicrobial activity but less effective than chlorhexidine. The 2% chlorhexidine group again demonstrated near-total microbial inhibition, with CFU values mostly being zero or one. Normal saline showed the highest CFU counts, ranging from 806 to 836, indicating negligible antimicrrobial action.

For *C. albicans*, the CFU after treatment with fennel seed extract ranged from 340 to 398, reflecting some antifungal activity. The 2% chlorhexidine group demonstrated strong antifungal efficacy, with CFU ranging from 0 to 6, while normal saline again showed the highest CFU values, ranging from 962 to 993, indicating a lack of antifungal activity.

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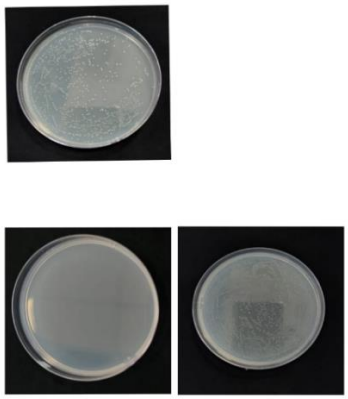


Figure 1-CFU of *E.faecalis*, post treatment with a. Fennel seed extract b. 2%CHX c.Normal saline

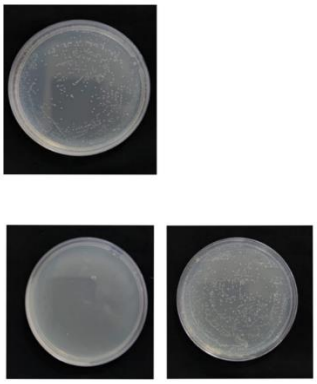


Figure 2- CFU of *S.aureus* - a. fennel seed extract b.2%CHX c. Normal saline(negative control)

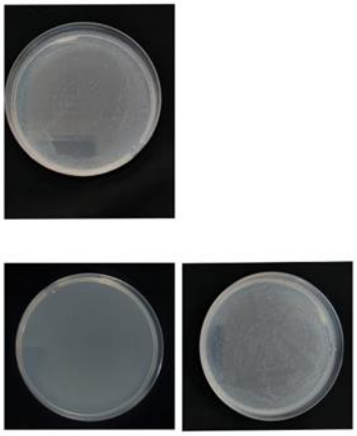
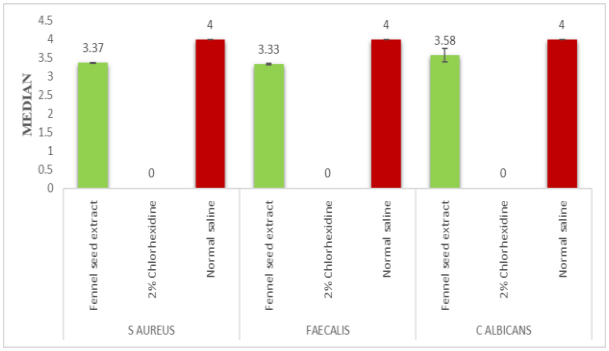


Figure 3-CFU of *C.albicans* - a. fennel seed extract b.2%CHX c. Normal saline(negative control)

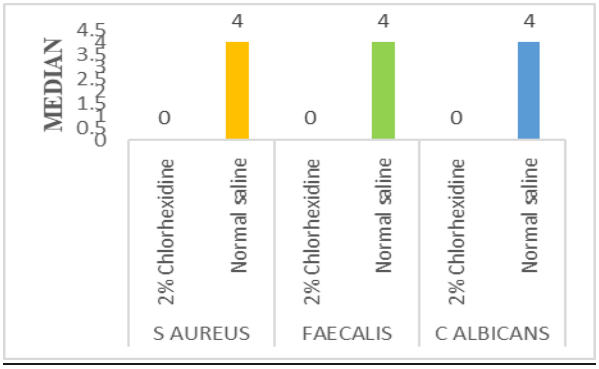
The Kruskal-Wallis H test was applied to assess the differences in antimicrobial efficacy of fennel seed extract across the three microorganisms (*E. faecalis*, *S. aureus*, and *C. albicans*). The test revealed a statistically significant difference ($p < 0.001$), indicating that fennel seed extract's antimicrobial effectiveness varies significantly depending on the microorganism tested.

Table 1:- : Antimicrobial efficacy of Fennel Seed Extract On Microorganism Growth

GROUP	Median	Mean	SD	IQR	Min	Max	Test Statistics	P value
E. FAECALIS	3.33	3.33	0.02	0.007	3.3	3.38	17.36	<0.001
S. AUREUS	3.37	3.37	0.01	0.017	3.36	3.39		
C.ALBICANS	3.58	3.52	0.18	0	3	3.6		



Graph 1 :- Graphical representation of antimicrobial efficacy of fennel seed extract against S.aureus, E.faecalis and C.albicans



Graph 2 :- graphical representation of antimicrobial efficacy of 2 % chlorhexidine against *E.faecalis, S.aureus and C.albicans*

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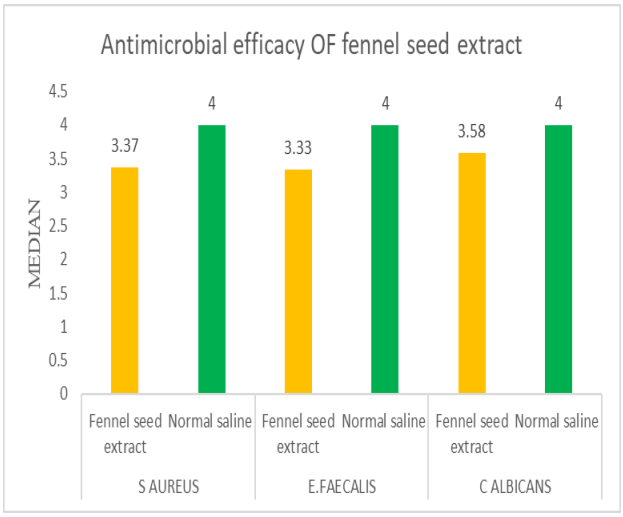

Graph 3 :-comparison of antimicrobial efficacy of fennel seed extract to that of 2 % chlorhexidine

When evaluating the antimicrobial efficacy of 2% chlorhexidine and normal saline, it was observed that chlorhexidine consistently achieved complete microbial inhibition across all tested microorganisms, with a median and mean CFU of zero and a standard deviation of zero. Normal saline consistently showed high microbial counts, with median and mean values of four.

Table 2: Antimicrobial Efficacy of 2% Chlorhexidine Against Microbial Groups

Groups	Variables	Median	Mean	SD
S.AUREUS	2% Chlorhexidine	0	0	0
	Normal saline	4	4	0
E.FAECALIS	2% Chlorhexidine	0	0	0
	Normal saline	4	4	0
C.ALBICANS	2% Chlorhexidine	0	0	0
	Normal saline	4	4	0

A direct comparison between fennel seed extract and 2% chlorhexidine using the Kruskal-Wallis H test showed statistically significant differences ($p < 0.001$) in antimicrobial efficacy for *E. faecalis*, *S. aureus*, and *C. albicans*. Chlorhexidine was markedly more effective than the fennel seed extract in inhibiting microbial growth.

Table 3: Comparison of Antimicrobial Efficacy Between Fennel Seed Extract and 2% Chlorhexidine

`Group	Variables	Mean	Median	SD	IQR	Min	Max	p value
S.AUREUS	Fennel seed extract	3.37	3.37	0.01	0.017	3.36	3.39	<0.001
	2% Chlorhexidine	0	0	0	0	0	0	
	Normal saline	4	4	0	0	4	4	
E.FAECALIS	Fennel seed extract	3.33	3.33	0.02	0.007	3.3	3.38	<0.001
	2% Chlorhexidine	0	0	0	0	0	0	
	Normal saline	4	4	0	0	4	4	
C.ALBICANS	Fennel seed extract	3.52	3.58	0.18	0	3	3.6	<0.001
	2% Chlorhexidine	0	0	0	0	0	0	
	Normal saline	4	4	0	0	4	4	

Discussion

Root canal irrigation plays a vital role in eliminating bacteria, dissolving necrotic tissue, and removing the smear layer to enhance obturation sealing [23]. NaOCl is widely used due to its strong antibacterial and tissue-dissolving properties but has several drawbacks, including cytotoxicity, unpleasant taste, and inability to remove the smear layer[23,9]. Chlorhexidine (CHX), a bisbiguanide disinfectant, offers prolonged antimicrobial activity due to its substantivity but lacks tissue-dissolving and smear layer removal capabilities[10,24].

Herbal remedies have gained interest in endodontics due to concerns about synthetic irrigants[25,26,19]. Tabassum Afshan et al. (2020) demonstrated neem's efficacy as an alternative irrigant[27], while Durga Bhavani et al. showed antimicrobial activity of triphala, propolis, and aloe vera against *E. faecalis*[28].

Fennel seed extract contains bioactive compounds like linoleic acid, anethole, and 5-hydroxyfuranocoumarin, contributing to antibacterial and antifungal properties against *E. faecalis*, *S. aureus*, and *C. albicans* [29,30,31]. However, its application as an irrigant remained unexplored, prompting this study. The aqueous extract was obtained by reflux method due to its higher yield and cost-effectiveness[32], with a concentration of 100 mg/ml used based on previous research [30].

The study used dentinal shavings for CFU assessment, providing accurate microbial quantification. Fennel extract significantly reduced *S. aureus* load (mean log

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CFU = 3.37, SD = 0.01) compared to saline (4.00) and CHX (0.00) ($p < 0.001$) . Against *E. faecalis*, known for its resistance, fennel showed measurable activity (mean log CFU = 3.33, SD = 0.02), with CHX achieving complete inhibition ($p < 0.001$).

For *C. albicans*, fennel extract showed a mean log CFU of 3.52 (SD = 0.18), with statistically significant reduction compared to saline ($p < 0.001$), though C. albicans displayed higher resistance, consistent with Mithun et al[33].

Normal saline showed no antimicrobial effect (mean and median CFU = 4.00, SD = 0.00), serving only as a mechanical irrigant.

Percentage inhibition by fennel seed extract was 17.5% (*E. faecalis*), 15% (*S. aureus*), and 10% (*C. albicans*), while CHX achieved 100% inhibition.

In conclusion, fennel seed extract demonstrated significant antimicrobial activity but was less effective than 2% CHX ($p < 0.001$). With growing interest in plant-based biocompatible alternatives, fennel extract shows promise for patients sensitive to chemical irrigants. Further in vivo studies are needed to determine optimal concentration and exposure time.

Limitations

This study was conducted in vitro using single-rooted teeth with a single canal, which does not represent the full anatomical variations encountered in clinical practice, such as severely curved roots, multi-rooted teeth, and multiple canals. The presence of isthmuses and difficulty in retrieving bacterial specimens from areas other than the main canal also limit the study's applicability.

Additionally, only a single concentration (100 mg/ml) of fennel seed extract was tested, though antimicrobial efficacy may vary with concentration. Modern irrigation techniques involving sonic or ultrasonic irrigators were not employed, which may influence the effectiveness of irrigant delivery and microbial removal.

Conclusion

Within the limitations of this study, it can be concluded that fennel seed extract exhibits antibacterial and antifungal activity against *E. faecalis*, *S. aureus*, and *C. albicans*, demonstrating potential as a plant-derived endodontic irrigant. However, its antimicrobial efficacy is significantly lower compared to 2% chlorhexidine. Further research is warranted to explore the synergistic effects of fennel seed extract with existing irrigants, and to optimize its concentration and formulation for enhanced antimicrobial efficacy and future clinical application.

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Conflict of interest

The authors have stated explicitly that there are no conflict of interest in connection with this article

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