Evaluation of antimicrobial activity of fennel seed extract with 2% chlorhexidine and 3% sodium hypochlorite against enterococcus faecalis as a root canal irrigant: an invitro study

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ABSTRACT

Introduction: The success of an endodontic treatment depends on effective disinfection and complete sealing of root canal. The irrigants that are currently used in the field of endodontics have certain limitations, so the quest for an ideal root canal irrigant continues. Nowadays, the use of herbal extracts such as Fennel seed extract is used for their potent antimicrobial activity and less side effects.

Objective: This study aimed to evaluate the antimicrobial efficacy of fennel seed extract with 2% chlorhexidine and 3% sodium hypochlorite against Enterococcus faecalis.

Materials and method: The antimicrobial efficacy of Fennel seed extract was analysed with different concentration (50,100mg/ml) with 2% chlorhexidine and 3% sodium hypochlorite using the agar well-diffusion method. Minimum inhibitory concentration of the active extract was determined by the broth dilution assay.

Result: Fennel seed extract at 50 and 100 percent concentrations is somewhat more effective against microbes than Sodium hypochlorite and chlorhexidine at 3% and 2%, respectively.

Conclusion: When compared to sodium hypochlorite and chlorhexidine, fennel seed extract exhibits somewhat greater antibacterial effectiveness against E. faecalis within the limitations. Hence, fennel seed extract shows promise as an irrigant for teeth.

Keywords: Anti-microbial activity, Enterococcus faecalis, Fennel seed.

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INTRODUCTION

Root canal treatment is an endodontic therapy aimed to save teeth that is severely infected or damaged to the pulp chamber and root canals1. The root canal process

entails meticulously removing decay, cleaning, shaping, and disinfecting the canal system, and then sealing it to avoid more infection2. It also alleviates pain, restores function and prevent the need of extraction.

Pulp and periapical disorders are mostly caused by microorganisms. Consequently, the main objective of root canal treatment is to eradicate all bacteria within the root canal3. Mechanical preparation is the main technique for reducing root canal disease and followed by intracanal irrigation which reduces the bacterial load. Enterococcus faecalis is the important and common bacterial species4. Tooth periodontitis and instances of botched root canal treatment also result in its isolation.

The introduction of chemical agents as intracanal irrigant helps in eradication of the microorganism. The most popular and often used root canal irrigant is sodium hypochlorite. A broad variety of microbes, including bacteria, viruses, and fungi, are susceptible to its powerful antimicrobial effects5. It may break down any remaining organic matter in the root canal. The process of producing hypochlorous acid (HOCl) upon interaction with organic waste is what makes it effective. Oxidising the sulfhydryl groups in the bacterial enzyme system is how HOCl exerts its action. Hence it disrupts the metabolism of bacteria and causes bacterial cell death6. Sodium hypochlorite also has certain side effects such as tissue toxicity, allergy, disagreeable smell and taste7. A wide variety of microbes may be killed by chlorhexidine (CHX). Additionally, it proves that it is substantial. In low concentration, CHX has bacteriostatic action and high concentration it has bactericidal action8. While it does mitigate sodium hypochlorite's drawbacks, it is unable to dissolve organic substances. Tooth discolouration, changed taste, and the development of antibiotic resistance are some of its negative aspects9.

Traditional remedies are all the rage again as a result of antibiotic resistance and their negative consequences. Herbal irrigants are advantageous because to their inexpensive price, extended shelf life, and absence of adverse effects.

Fennel seed extract possesses anti-inflammatory and anti-bacterial action. One component of fennel's antibacterial effect is 5-hydroxy-furanocoumarin, which is found in the plant's seeds10.

MATERIALS AND METHODS

Solutions containing 2% chlorhexidine and 3% sodium hypochlorite were used for an in vitro microbiological experiment involving fennel seed extract at concentrations of 50 mg/ml and 100 mg/ml, respectively. The antibacterial activity against E. faecalis was tested using the agar well diffusion technique.

Preparation Of Extract

The fennel seed was ground into a fine powder to make the extract used in the research. Two separate solutions were made with fennel seed extract. In the first tube, 50mg of the fennel seed extract powder with 10ml of distilled water is mixed . This creates a 50mg/mL concentration. In the second tube, 100mg of the fennel seed extract powder with another 10ml of distilled water is mixed . This creates a 100mg/mL concentration. A thermostatic water bath is used to insert the centrifuge tubes with the two extract solutions. For 20 minutes, the water bath is kept at a temperature of 60°C. The next step is to let the solutions cool to room temperature. After that, for 10 minutes, the solutions are spun in a centrifuge at 2500 rpm.

Agar Well Diffusion Method

To isolate E. faecalis colonies, a pure culture is streaked onto a Mueller-Hinton agar plate and let to incubate at 37°C overnight. Overnight incubation at 37°C follows the transfer of several colonies into Mueller-Hinton broth using a sterile swab. The plates of sterile Mueller-Hinton agar are set up. A sterile 6 mm well punch is used to create wells in the solidified Mueller-Hinton agar media on each agar plate. The following solutions are loaded into separate wells on each plate: 50 µL of the 50mg/mL fennel seed extract solution. 50 µL of the 100mg/mL fennel seed extract solution. 50 µL of the 3% sodium hypochlorite solution. 50 µL of the 2% chlorhexidine solution. In order to guarantee that the E. faecalis broth culture is evenly distributed throughout all of the Mueller-Hinton agar plates, a sterile brush is used to inoculate the surface of each plate. To cultivate E. faecalis, all inoculation plates are set in an anaerobic jar, which is designed to provide an oxygen-depleted environment. Incubating the plates at 37°C for an additional 24 hours is the standard procedure. As soon as the incubation time is up, the plates are examined for any patches of transparency surrounding the wells. These regions show where the test solutions were able to prevent the development of bacteria. Using a standardised inhibition zone reader template, the diameter of each inhibition zone is meticulously measured.

Determination Of Minimum Inhibitory Concentration

A pure culture of Escherichia coli is streaked onto a Mueller-Hinton agar plate, and then left to incubate at 37°C overnight to obtain colonies. After that, a number of colonies are moved into Mueller-Hinton broth and let to incubate at 37°C for the night. A turbidity of (1.5 x 108 CFU/mL) may be achieved by standardising the overnight culture. In sterile glass tubes, using Mueller-Hinton broth, make two serial dilutions of the stock extract solution. To each sterile glass tube containing 0.5 mL of Mueller-Hinton broth with a specific extract concentration, add 2 mL of the standardised E. faecalis inoculum using a micropipette. Put the tubes in an anaerobic container and set the temperature to 37°C for 18 to 24 hours to promote the growth of E. faecalis. Due to their turbidity and dark colour, the extracts were mixed with Mueller-Hinton agar and put to a plate with a single layer of 0.1 ml capacity. Incubation at 37°C for 18-24 hours follows inoculation of the Mueller-Hinton agar plates.

Statistical Analysis

The minimal inhibitory concentration of fennel seed extract was determined using a t test, which ranged from 50 to 100 mg/ml. In order to determine the inhibition zone, a one-way analysis of variance test was used. The level of significance, $P<0.05$, was chosen.

RESULTS

The mean values across the experimental group were significantly different at $p<0.05$. Fennel seed extract at 50 and 100 percent concentrations is somewhat more effective against microbes than Sodium hypochlorite and chlorhexidine at 3% and 2%, respectively (Table 1) (Figure 2). The minimal inhibition concentration of 100 mg/ml Fennel seed extract is lower than the 50mg/ml of fennel seed extract (Table 2) (Figure 1).

DISCUSSION

The goal of this study was to find out whether enterococcus faecalis could be effectively killed by endodontic irrigation using fennel seed extract. Antibacterial efficacy of 50 and 100 % fennel seed extract is marginally higher than that of 3% sodium hypochlorite and 2% chlorhexidine. The minimal inhibition concentration of 100 mg/ml Fennel seed extract is lower than the 50mg/ml of fennel seed extract. The capacity of E. faecalis to form biofilms in medicated root canals and to cause reinfection after obturation is the reason for its selection11. Sodium hypochlorite is one of the agents that has been used extensively over the years to disinfect root canals. Unspecific proteolytic agent that dissolves the remaining pulp tissue and has antibacterial qualities12.Higher concentrations of sodium hypochlorite are employed to eradicate the germs, effectively getting rid of the bugs. Additionally, it results in a decrease in dentin's flexural strength and elastic modulus. It has an indirect impact on the root canal dentin's structural integrity13.

Chlorhexidine (CHX) exhibits substantivity and a wide range of antibacterial action14. As CHX penetrates the outer membrane of microbes, it has an effect on their cytoplasmic or inner membranes, as well as their plasma membranes. CHX causes intracellular components to coagulate when concentrations are high15. The fact that CHX attaches to hard tissue and maintains its antibacterial properties is one of the factors contributing to its substantivity, or ongoing antimicrobial impact. As with other endodontic disinfectants, the effectiveness of CHX is pH-dependent and drastically reduced when organic components are present16.

People are increasingly seeking out traditional herbal remedies as an alternative to synthetic antibiotics due to concerns about the increasing number of germs and viruses that are resistant to these treatments, as well as their negative effects. Herbal irrigants are advantageous due to their short shelf life, low cost, lack of microbiological resistance, safety, ease of availability, and convenience17.

Fennel (Foeniculum vulgare) which is a spice found largerly in Tropical region. It is one of the significant herbal plant that has important medicinal qualities. It has phenolic chemicals which is important to human health18. Fennel contains 5-hydroxy-furanocoumarin, an active ingredient in the plant that has antimicrobial properties. The watery extract of fennel has bactericidal properties against E. faecalis19.

The antibacterial activity of several groups has already been assessed using the more practical, dependable, and generally acknowledged agar well-diffusion technique20.

We evaluated the aqueous extract's antibacterial activity against Enterococcus faecalis at different concentrations; using other solvents, such as alcohol, will modify the efficacy of the herbs' antibacterial activity since those solvents will dissolve the herbs more easily. Because of potential variations in the plant's efficacy, even in vitro results might not be entirely transferable to the clinical setting.

CONCLUSION

When compared to sodium hypochlorite and chlorhexidine, fennel seed extract exhibits somewhat

greater antibacterial effectiveness against E. faecalis within the limitations. Hence, fennel seed extract shows promise as an irrigant for teeth.

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TABLE : 1

Timeline	t statistic	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
				Lower	Upper
24 hours	117.874	.0001	.212500	.204743	.220257

Timeline	Group	Mean	Std. Deviation	Std. Error Mean
HOUR 24	50 conc	.36550	.002121	.001500
	100 conc	.15300	.001414	.001000

Group	Mean	Std.Deviation	95% Confidence		P Value
			Lower Bound	Upper Bound	
50 conc Fennel seed extract	15.5000	.70711	9.1469	21.8531	0.026
100 conc Fennel seed extract	16.5000	.70711	10.1469	22.8531	
2% CHX	12.5000	.70711	6.1469	18.8531	
3% NaOCl	13.0000	.00000	13.0000	13.0000	

FIGURE : 1 MINIMUM INHIBITORY CONCENTRATION

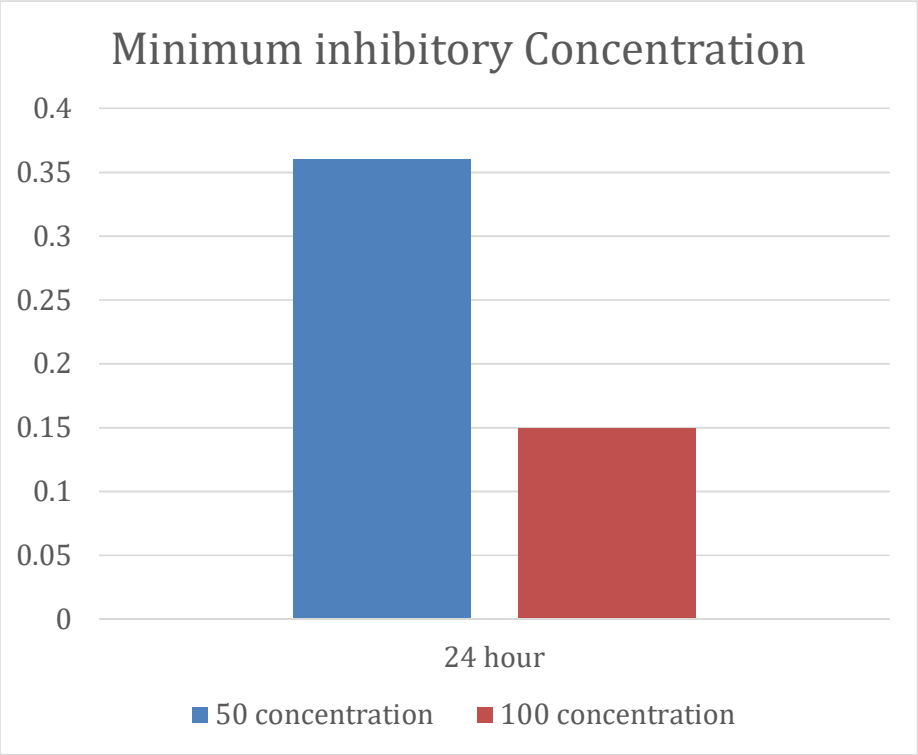

FIGURE : 2 ZONE OF INHIBITION