Original Article

Antimicrobial activity of Five Different Essential oils against Enterococcus Faecalis: An In vitro study

Disha Bansal [1], Iva Chandola [2], Mrinalini Mahajan[3]

[1] Associate Professor, Department of Dentistry, Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, India

[2] Assistant Professor, Department of Microbiology, Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, India

[3] Professor and Head, Department of Dentistry, Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, India

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Abstract

Introduction: Refractory root canal infection is mostly associated with enterococcus faecalis. The chemomechanical cleaning of root canal is one of the most critical steps in endodontic treatment. Intracanal medicaments are used as a supplementary disinfection process. Essential oils are rich in antibacterial properties and can be used against bacteria in root canals.Aim of this study was to evaluate antimicrobial activity of 5 essential oils mixed with calcium hydroxide against E.faecalis **Methods:** Enterococcus faecalis (ATCC 29212) was assigned as test organism, inoculated into Brain heart infusion broth, incubated overnight at 37^0 C and subcultured onto Brain heart infusion agar. 4 cup wells of 10 mm diameter were bored in each petriplate. These wells were then filled with freshly prepared test medicaments and incubated for 24 hours in upright position. The zones of inhibition were analyzed and diameters were measured using a ruler**. Results:** The mean zone of inhibition was significantly higher among Geranium oil + Ca(OH)2, Lemon grass oil+ Ca(OH)2, Rosemary oil+ Ca(OH)2 and Saline + Ca(OH)2 when compared to Jojoba oil +Ca(OH)2 and Almond oil+ Ca(OH)2.**Conclusion:**Calcium hydroxide combined with essential oils can be used as an effective intracanal medicament against E.faecalis.

Keywords: Essential oils, Antimicrobial, E. faecalis, Zone of inhibition

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Introduction

The main purpose of chemomechanical preparation is to reduce microbial load in root canals. Thorough removal of microbial load from root canal is a challenging task due to accessory canals and dentinal tubules. (1, 2) Use of intracanal medicaments have been proposed to help achieving a complete disinfection of root canals. Intracanal medicaments would help to remove the remaining bacteria and their byproducts in root canals. (1, 2)

The most common bacteria related to peri-apical lesions refractory to endodontic treatments is Enterococcus faecalis. This bacteria might cause failure due to its resistance to some known intracanal medicaments.

Since E.faecalis is resistant to difficult conditions like starvation and high PH, it can easily survive in root canals (3). Further, it can survive in the root canal without any synergism with other organisms (4).

None of the traditional intracanal medicaments have shown inhibition and recolonization of all types of bacteria in root canals. Most of these medicaments are cytotoxic in nature and can lead to irritation of periradicular tissue (5). Hence, there is a persistent search to discover new materials which would be less cytotoxic to periradicular tissue while possess acceptable antimicrobial properties (6). Natural derivatives or herbal solutions have been applied in medical and dental practice since many years ago due to their superior characteristics. These properties include antimicrobial activity, lack of bacterial resistance, biocompatibility, anti-inflammatory or antioxidant, ease of access and low cost (7).

The aim of this study was to evaluate in-vitro antibacterial properties of 5 different essential oils mixed with calcium hydroxide on Enterococcus faecalis.

Materials and Methods

Enterococcus faecalis (ATCC 29212) was assigned as the test organism. *E. faecalis* was inoculated into Brain heart infusion (BHI) broth, incubated overnight at 37^0 C and subcultured onto Brain heart infusion agar. The inoculum of colonies from BHI agar was further plated onto bile esculin agar for confirmation of pure culture of *E. faecalis*, seen as pinpoint black colonies. 4- 5 colonies of this pure culture of Enterococcus faecalis from BHI agar were added to 10 ml sterile distilled water and turbidity was adjusted to 0.5 McFarland opacity standard. Antibacterial efficacy tested on BHI agar, cup well agar diffusion method was used in which 30 ml culture medium was dispensed in respective petridishes which were inoculated with 0.1 ml fresh culture of 0.5 McFarland *E. faecalis*. 4 cup wells of 10 mm diameter were bored in each petriplate. These wells were then filled with the freshly prepared test agent. Each test agent group assayed in 12 replicates.

Groups descriptions were as below:

Group 1: Jojoba oil (Aruba essentials Brand) + Calcium hydroxide

Group 2: Geranium oil (Aruba essentials) + Calcium hydroxide

Group 3: Lemongrass oil (Aruba essentials) + Calcium hydroxide

Group 4: Almond oil (Aruba essentials) + Calcium hydroxide

Group 5: Rosemary oil (Aruba essentials) + Calcium hydroxide

Group 6: Saline + Calcium hydroxide (Control)

All the inoculated media plates were incubated for 24 hours at 37^0 C under aerobic conditions. Clear zones surrounding each sample indicative of bacterial growth inhibition were measured using a ruler and recorded as diameter of complete growth inhibition.

Results

Table I: Comparison of different groups clear zones

	Zone of inhibition					
	Mean±SD	95% Confidence Interval		Minimum	Maximum	F-value	p-value
Jojoba oil +Ca(OH)2	10.00±0.00	10.00	10.00	10.00	10.00		
Geranium oil + Ca(OH)2	25.25±0.45	24.96	25.54	25.00	26.00		
Lemon grass oil+ Ca(OH)2	26.08±0.51	25.76	26.41	25.00	27.00		
Almond oil+ Ca(OH)2	10.00±0.00	10.00	10.00	10.00	10.00		
Rosemary oil+ Ca(OH)2	28.83±0.39	28.59	29.08	28.00	29.00	6,506.047	< 0.001*
Saline + Ca(OH)2	22.83±0.39	22.59	23.08	22.00	23.00		

One-way ANOVA test * Significant difference

The mean zone of inhibition was compared between different groups; Jojoba oil +Ca(OH)2 (Fig. 1), Geranium oil + Ca(OH)2 (Fig. 2), Lemon grass oil+ Ca(OH)2 (Fig. 3), Almond oil+ Ca(OH)2 (Fig. 4), Rosemary oil+ Ca(OH)2 (Fig. 5) and Saline + Ca(OH)2 (Fig. 6) using one-way ANOVA test (Table I) (Graph I).

There was a significant difference in mean zone of inhibition between Jojoba oil +Ca(OH)2, Geranium oil + Ca(OH)2, Lemon grass oil+ Ca(OH)2, Almond oil+ Ca(OH)2, Rosemary oil+ Ca(OH)2 and Saline + Ca(OH)2.

Figure 1: Zone of inhibition for Jojoba oil + $Ca(OH)_2$

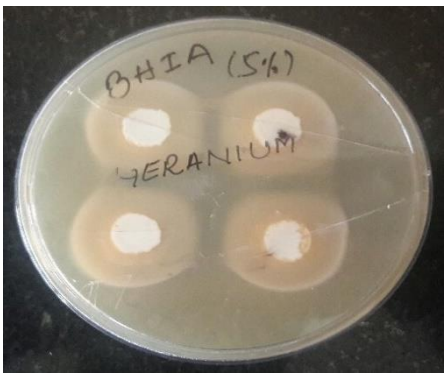


Figure 2: Zone of inhibition for Geranium oil + $Ca(OH)_2$

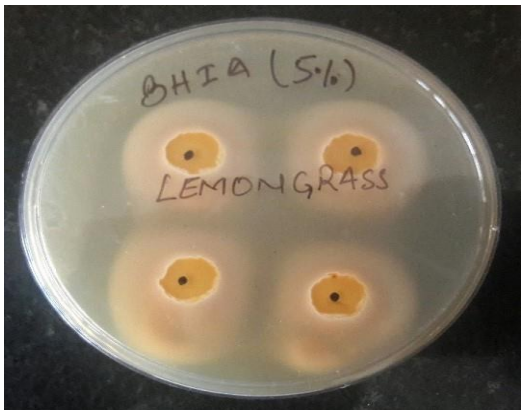


Figure 3: Zone of inhibition for Lemongrass oil + $Ca(OH)_2$

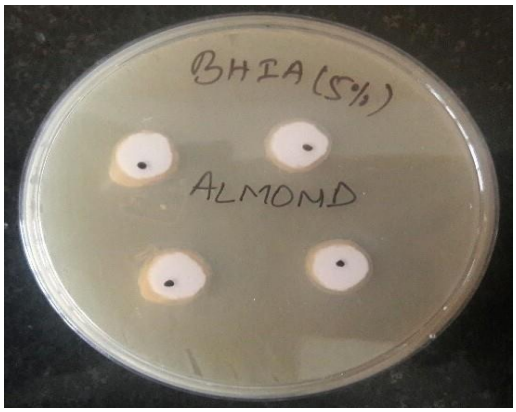


Figure 4: Zone of inhibition for Almond oil + $Ca(OH)_2$

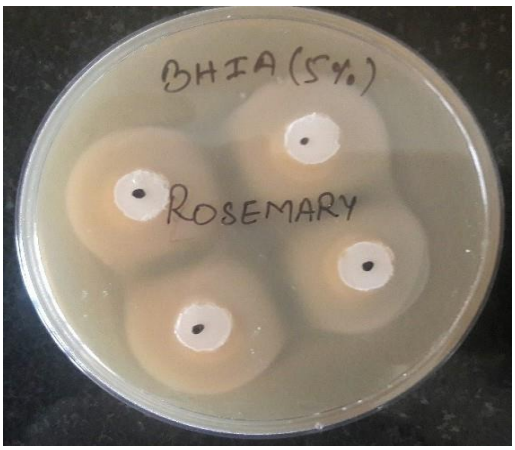


Figure 5: Zone of inhibition for Rosemary oil + $Ca(OH)_2$

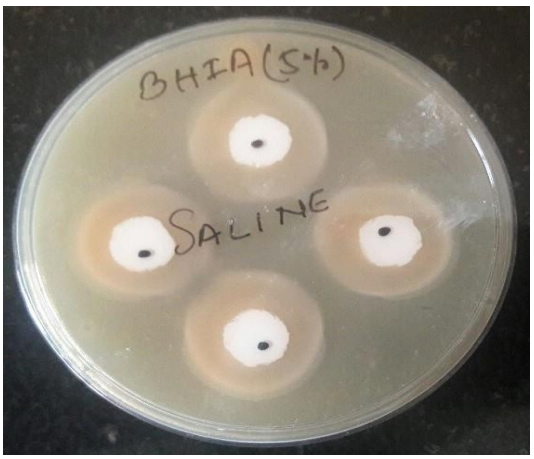
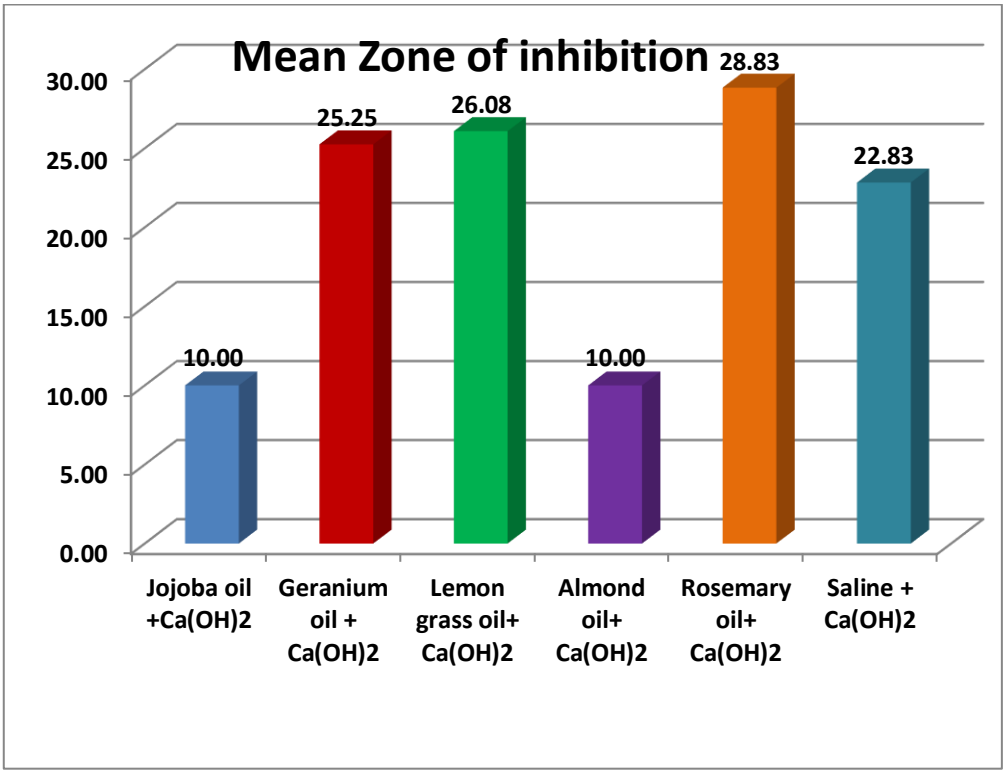


Figure 6: Zone of inhibition for Saline + $Ca(OH)_2$



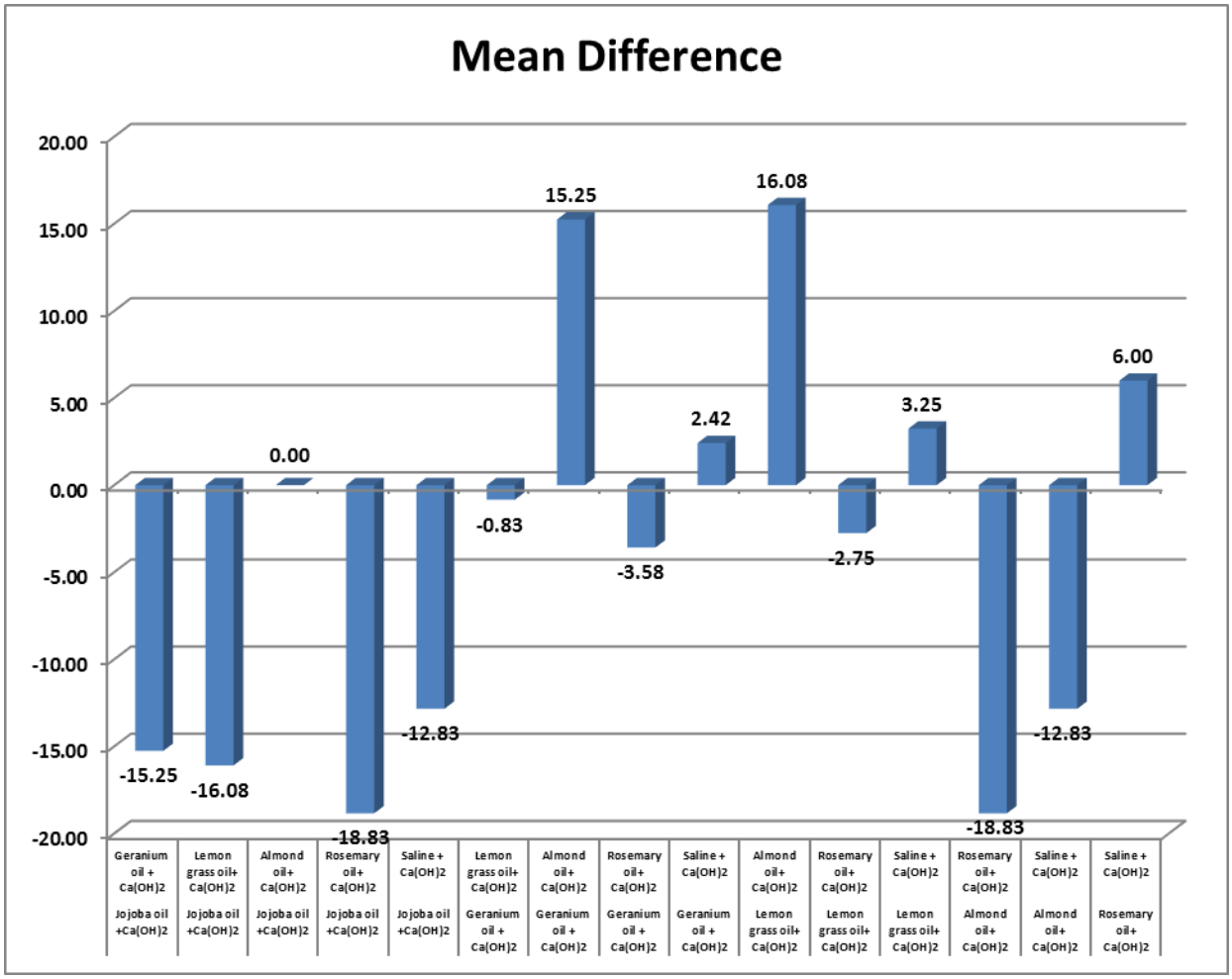
Graph I: Comparison of inhibition zone of test agents.

Table II: Comparison of Mean Difference between zones of inhibition

		Mean difference	p-value
Jojoba oil +Ca(OH)2	Geranium oil + Ca(OH)2	-15.25	< 0.001*
Jojoba oil +Ca(OH)2	Lemon grass oil+ Ca(OH)2	-16.08	< 0.001*
Jojoba oil +Ca(OH)2	Almond oil+ Ca(OH)2	0.00	1.000
Jojoba oil +Ca(OH)2	Rosemary oil+ Ca(OH)2	-18.83	< 0.001*
Jojoba oil +Ca(OH)2	Saline + Ca(OH)2	-12.83	< 0.001*
Geranium oil + Ca(OH)2	Lemon grass oil+ Ca(OH)2	-0.83	< 0.001*
Geranium oil + Ca(OH)2	Almond oil+ Ca(OH)2	15.25	< 0.001*
Geranium oil + Ca(OH)2	Rosemary oil+ Ca(OH)2	-3.58	< 0.001*
Geranium oil + Ca(OH)2	Saline + Ca(OH)2	2.42	< 0.001*
Lemon grass oil+ Ca(OH)2	Almond oil+ Ca(OH)2	16.08	< 0.001*
Lemon grass oil+ Ca(OH)2	Rosemary oil+ Ca(OH)2	-2.75	< 0.001*
Lemon grass oil+ Ca(OH)2	Saline + Ca(OH)2	3.25	< 0.001*
Almond oil+ Ca(OH)2	Rosemary oil+ Ca(OH)2	-18.83	< 0.001*
Almond oil+ Ca(OH)2	Saline + Ca(OH)2	-12.83	< 0.001*
Rosemary oil+ Ca(OH)2	Saline + Ca(OH)2	6.00	< 0.001*

Post-hoc bonferroni test * Significant difference

The inter-group comparison of mean zone of inhibition was done using the Post-hoc bonferroni test (Table II) (Graph II). The mean zone of inhibition was significantly more among Geranium oil + Ca(OH)2, Lemon grass oil+ Ca(OH)2, Rosemary oil+ Ca(OH)2 and Saline + Ca(OH)2 compared to Jojoba oil +Ca(OH)2 and Almond oil+ Ca(OH)2.



Graph II: Intergroup Comparison of Mean Difference between Zones of Inhibition.

Discussion

The main advantages of herbal and natural alternatives in endodontics are lack of microbial resistance, biocompatibility, ease of access and low cost (8).

A facultative gram positive anaerobe Enterrococcus faecalis is a well-studied cause of endodontic refractory infection (9). As it is the known pathogen which is usually in association with the apical periodontitis in the teeth that is previously endodontically treated. That is why, it is considered as the best organism to study antimicrobial activity of intracanal medicament (10).

Numerous properties of intracanal medicaments such as antimicrobial action, tooth resorption inhibition, tissue dissolving ability and formation of hard tissue have already been found and investigated and its usage in dentistry and endodontics has been related to healing of periradicular tissue and some unfavourable reactions (11).

According to World Health Organization (WHO), almost 80% of the population of world is dependent on traditional (herbal) medicine for their healthcare needs at primary level (12).

Plants have been a source of medicinal compounds and always played a major role in maintaining a healthy condition in human beings (13).

The hydrophobicity of essential oils and its components disturb bacterial cell walls making them permeable by partitioning the lipid content of the cell membrane and mitochondria (14). Leakage from the wall of the cell will lead to death of bacteria due to loss of critical molecules and ions (15).

The most common screening method to measure antimicrobial efficacy of Essential oils, medicinal plants and their constituents is Agar gel diffusion test (16).

Folk medicines contain different jojoba plant extracts, Jojoba is also known as a food preservative. Jojoba oil has shown antibacterial action against E.coli, Staphylococcus aureus, Klebsiella pneumonia and Pseudomonas aeruginosa in previous studies (13). In this study, Jojoba oil abort any antibacterial activity against E. faecalis.

Dorian et al showed growth of antibiotic resistant bacteria, were inhibited by essential oil vapors. Various effects were observed depending on duration of exposure and testing environment (17).

The combination of Almond oil + calcium hydroxide powder and Jojoba oil + calcium hydroxide powder showed lesser activity against the test organism than Calcium hydroxide with saline. This indicates that antimicrobial activity of Almond oil and Jojoba oil with calcium hydroxide did not have a synergistic effect.

Rusenova et al stated that *Cymbopogon citratus* (lemongrass) has strong antimicrobial activity against C. Albicans (inhibition zone> 20mm) and moderate antimicrobial activity against E.faecalis (inhibition zone12-20mm) (18). This study showed significant activity of lemongrass oil and calcium hydroxide against the test organism.

Previous studies showed that Geranium oil has showed antibacterial action against the strains of Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 15380, Pseudomonas aeruginosa ATCC 27853, and Staphylococcus aureus ATCC 25923 (19). Continuing with this line of investigation, antimicrobial activity of geranium oil in addition to calcium hydroxide was studied against E.faecalis.

Previous studies showed an effect of inhibition of geranium essential oil against enterococcal strains of endodontic origin which had developed antibiotic sensitivity and drug resistance (20).This study showed results along with this previous study as Geranium oil in addition to calcium hydroxide showed significant activity against test organisms.

Rosmarinus officinalis, L. is known to be rich in compounds of phenol and the properties possessed, are derived from its extracts and the essential oils. These are used in the preservation of food and for the treatment of various diseases (21).

Inhibitory actions of rosemary are the result of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol and isorosmanol. These probably act on the cell membrane, cause alteration in genes and nutrition, altering transfer of electrons, leak components of cells and modify formation of fatty acids. As well, it interfere with proteins of membrane leading to loss of function and structure of it(22).This study shows significant antimicrobial action of Rosemary oil and calcium hydroxide in opposition of E.faecalis.

Evaluating antimicrobial action of essential oils in tandem with another strong antimicrobial material like calcium hydroxide could be discussed as limitation of this study. However, standardization of test agent was maintained during the study to overcome this limitation.

Conclusion

Essential oils like Rosemary, Lemongrass and Geranium together with Calcium hydroxide could be possible alternatives to other antimicrobial materials during endodontic treatment.

Conflict of Interest

Authors claim no conflict of interest.

Acknowledgment

There are no acknowledgements.

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Corresponding Author
Disha Bansal
Associate Professor, Department of Dentistry, Shri Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, India
Tell: 8979466863
Email: dishaendodontist@gmail.com