RESEARCH PAPER

Comparative Evaluation of Anti-Microbial Efficacy of 2% Chitosan, Dry Ginger Powder and Calcium Hydroxide in Additives

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ABSTRACT

Background: Complete disinfection of the root canal system is important for endodontic success. Chitosan, a biopolymer, has demonstrated antimicrobial properties. Dry ginger and calcium hydroxide are also known to possess antimicrobial activity. Their combined effect has not been extensively studied.

Aim: To assess and compare the antimicrobial efficacy of three intracanal medicament formulations: 2% chitosan gel, 2% chitosan with dry ginger powder, and 2% chitosan with dry ginger powder and calcium hydroxide.

Methods: Three test groups were prepared: Group 1 – 2% chitosan gel; Group 2 – 2% chitosan gel with 300 mg dry ginger powder; Group 3 – 2% chitosan gel with 150 mg dry ginger powder and 150 mg calcium hydroxide. Antimicrobial efficacy was tested against *Enterococcus faecalis*, *Streptococcus mutans*, and *Candida albicans* using the agar diffusion method (zone of inhibition) and serial dilution method (minimum inhibitory concentration, MIC). Data were analyzed using the Mann-Whitney U test.

Results: Group 3 showed the highest antimicrobial efficacy, with inhibition zones of 14 mm (*E. faecalis*), 13 mm (*S. mutans*), and 14 mm (*C. albicans*), and the lowest MIC values (down to 0.0005 mg/mL). Group 1 (chitosan alone) showed no measurable inhibition. Statistical analysis revealed significant differences between groups ($p < 0.05$).

Conclusion: The formulation combining 2% chitosan, dry ginger powder, and calcium hydroxide demonstrated superior antimicrobial properties and could be considered a promising intracanal medicament.

Keywords: *Chitosan, calcium hydroxide, dry ginger, antimicrobial activity, intracanal medicament, zone of inhibition, MIC.*

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INTRODUCTION

The oral cavity is one of the most vulnerable and dynamic environments in the human body, home to a diverse community of microorganisms. These include bacteria, fungi, protozoa, and viruses, many of which coexist as part of a balanced ecosystem.. However, when this balance is disturbed, it can lead to microbial overgrowth and cellular damage, ultimately affecting the health of the entire oral environment [1].

Some of the most implicated bacteria in oral infections—particularly those that lead to demineralization of the enamel and deeper dental structures—include *Streptococci*, *Peptostreptococcus*, *Lactobacilli*, *Propionibacterium*, and *Actinomyces*. These organisms not only affect the soft tissues like the gums and mucosa but also contribute to the breakdown of root surfaces and apical structures.

When the microbial balance is chronically disrupted, it can lead to dental caries, which, over time, progress through the enamel and dentin layers into the pulp, resulting in pulpitis. Because oral samples are relatively easy to collect, the study of oral microflora has significantly advanced our understanding of dental diseases. [2],[3].

In modern dentistry, one of the key goals is to eliminate pathogenic microorganisms that compromise the health of teeth and surrounding tissues. Toyoshima et al stated that the success of endodontic treatment heavily depends on the effective removal of these pathogens [4]. Alongside mechanical cleaning, intracanal medicaments play a vital role in disinfection of the canal and improving treatment outcomes. Over the years, many such agents have been introduced, each with unique antimicrobial properties. Ginger, a natural agent containing bioactive compounds such as gingerol and shogaol, also exhibits antimicrobial potential.

Chitosan, a naturally derived biopolymer from crustacean shells, has gained attention for its antimicrobial potential and is now available commercially in various forms [5].

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While calcium hydroxide remains a conventional choice in root canal therapy.

However, there is limited evidence on the combined use of chitosan, ginger, and calcium hydroxide as an intracanal medicament. Exploring this combination may reveal synergistic effects that could enhance antimicrobial efficacy against endodontic pathogens

This study aims to explore and compare the antimicrobial effectiveness of 2% chitosan gel, chitosan combined with dry ginger powder, and their combination with calcium hydroxide against common root canal pathogens.

Materials and Methods

Protocol

This study was conducted in the orange lab of Saveetha Dental College, deemed under Saveetha University Chennai. The study commenced after proper clearance from the ethical board of research and analytics, Saveetha Dental College. (SRB/SDC/PEDO-2303/24/250.) This study analyzes common microflora of the oral cavity such as Candida Albicans, S. Mutans, E. Faecalis and P. gingivalis.

Formulations:

- **Group 1:** 2% chitosan gel (made with 2 g chitosan in 100 mL acetic acid solution).
- **Group 2:** 2% chitosan + 300 mg dry ginger powder.
- **Group 3:** 2% chitosan + 150 mg dry ginger + 150 mg calcium hydroxide.

Microorganisms:

Four clinically relevant root canal pathogens were selected: *Streptococcus mutans*, *Enterococcus faecalis*, *Candida albicans*, and *Porphyromonas gingivalis*.

Antimicrobial Assays:

Agar well diffusion test was used to evaluate the zone of inhibition (ZOI) for all four microorganisms.

Minimum inhibitory concentration (MIC) was determined using broth dilution for *S. mutans*, *E. faecalis*, and *C. albicans.*

For *P. gingivalis*, only ZOI was measured due to its strict anaerobic requirements, making broth dilution MIC determination infeasible.

Replicates and Reproducibility:

All experiments were performed in triplicate (n = 3 per group per microorganism) to ensure reproducibility. Independent repeats were carried out on separate days to confirm consistency of results. Mean values and standard deviations were calculated from the replicate data.

Zone of inhibition and minimum inhibitory concentration

S.mutans was cultured in Mutans-Sanguis Agar, E. Faecalis was cultured in mueller hinton agar and Candida Albicans was cultured in Sabouraud dextrose agar. Each of the agar medium was inoculated with 10^6 of microorganism colonies respectively from each of the selected species. Wells were created in the agar medium to place the intracanal medicaments which were incubated at 37^0C for 24 hours. Once the set timeframe was achieved the agar plates were then observed for zones of inhibition around the wells using the antibiotic zones scale himedia, a tool used to measure the growth inhibition zone sizes of antimicrobial susceptibility test discs. (Figure 2)

MBC (Minimum Inhibitory Concentration) was analyzed for each of the species induced agar plates by serial dilution method. BHA broth was used for S. Mutans, and E. faecalis and Sabouraud dextrose broth was used for C. Albicans. 300µl of these broths were added to each of the wells in all the agar plates. The broths were respectively added into wells designed as control groups in all the agar plates which have no antibiotic or intracanal medicament loaded in the wells. (Figure 1)

Figure 1: Shows wells with intracanal medicaments to assess minimum inhibitory concentration at 24 hours for Streptococcus mutans, Candida albicans, Enterococcus faecalis respectively.

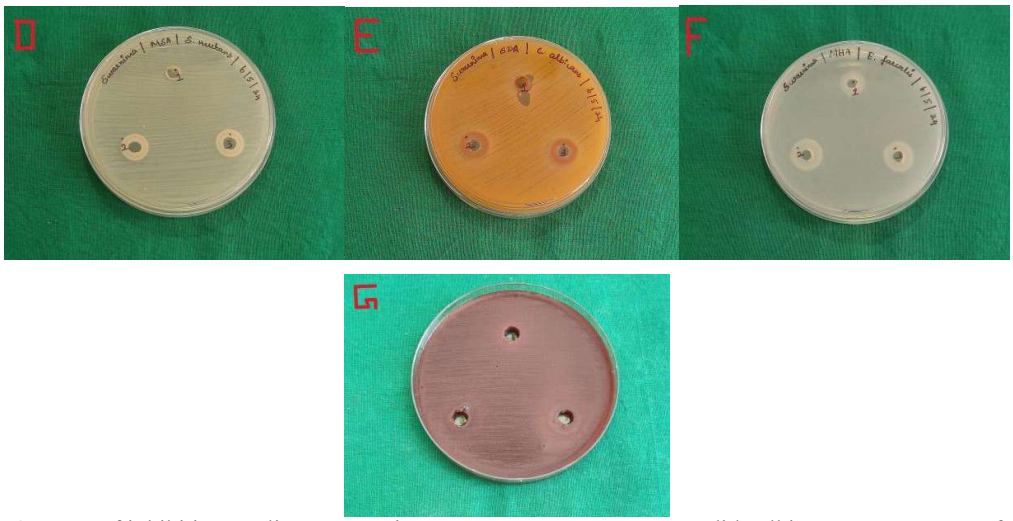

Figure 2: Zone of inhibition medicament against Streptococcus mutans, Candida albicans, Enterococcus faecalis, Porphyromonas gingivalis respectively.

The readings were taken by robonik elisa reader after the agar plates were left for 24 hours in 37^0C at 450 nm. The calculation for the decreasing colonies were done by subtracting the values obtained by testing the antibiotic groups from the Control group. The percentage value was also obtained by dividing test values by control values which was multiplied by 100 units.

The procedures and protocol differed for P. Gingivalis as it is an anaerobic bacterium. Anaerobic blood agar plates were employed for culturing P. Gingivalis and wells created in the plate are similar to earlier designs of wells created for aerobic microorganisms. The plate was then introduced into anaerobic gas jars for a total of seven days. The reading was done after seven days by the same method that was used for aerobic microorganisms. Only the zone of inhibition was calculated for P.gingivalis. As *P. gingivalis* is an obligate anaerobe, MIC determination using broth dilution was not feasible due to its growth requirements. Instead, only the zone of inhibition was measured.

STATISTICAL ANALYSIS

The acquired numerical values for each of the cultures in different concentrations were sorted in terms of zones of inhibition in the microsoft excel sheet. The sorted data was analyzed statistically using SPSS (version 26). One way ANOVA was done to observe the statistical significance for the zone of inhibition.

RESULTS

The results obtained from strictly adhering to the protocols planned out for this study was considered significant (p value<0.05) in terms of antimicrobial activity of the selected microflora towards the sequence of intra canal medications mentioned above. Ginger powder extract shows a synergistic effect which is clearly reflected in the data obtained depicted in Figure 3. The combination of all three additives, 2% chitosan gel, ginger powder extract calcium hydroxide powder gave promising results when compared to only combining 2% chitosan gel and ginger powder extract.

The ZoI(Zone of Inhibition) was 16mm on blood agar plates infused with P. Gingivalis around the well of additives of all three intracanal medicaments. P. Gingivalis was more sensitive to the intracanal medicaments that were subjected onto the bacterial colonies.Minimum inhibitory concentration was not calculated for P.gingivalis. In summary, the overall bactericidal potential of calcium hydroxide drastically increased when used in addition with 2% chitosan gel and ginger powder extract Whereas when chitosan used alone cannot be relied upon as an effective antimicrobial agent.

Table 1 and Table 1.1 gives us the values for zone of inhibition and mean value with standard deviation and p value for zone of inhibition for the mentioned organisms and samples respectively. Table 2 gives us data regarding the minimum inhibitory concentration for the mentioned organisms.

Table 1 : Values of zone of inhibition

Zone of inhibition	Sample 1(mm)	Sample 2(mm)	Sample 3(mm)
S.Mutans	0	12	13
E.Faecalis	0	10	14

C.Albicans	0	14	14
P.Gingivalis	8	9	16

Table 1.1: Mean value for zone of inhibition and P value

	Sample 1	**Sample 2**	**Sample 3**
MEAN VALUE	1.6	9	11.4
STD.DEV	3.5777	5.3852	6.4653
P-value	0.0314		

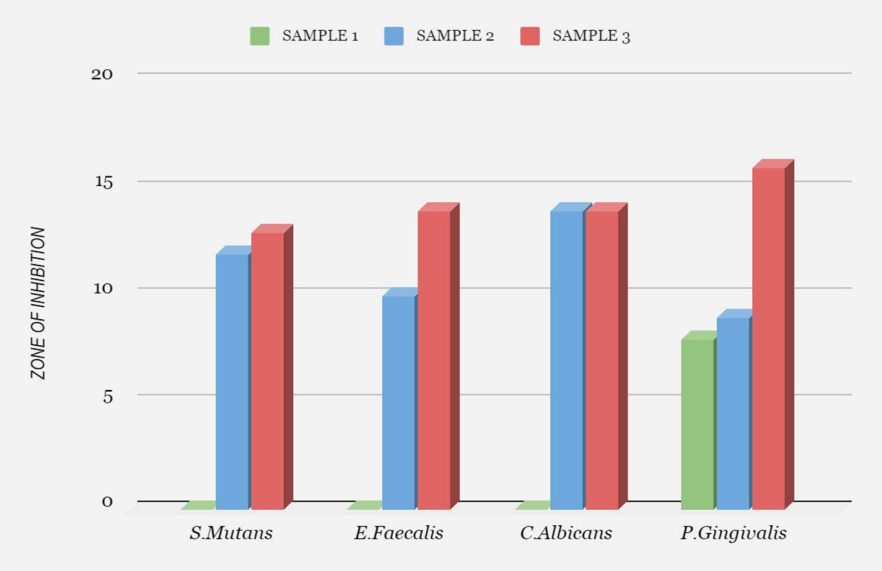

Figure 3: X axis represents the zone of inhibition of colonies of microflora cultured in a controlled environment. Y axis denotes the Zone of Inhibition for the above-mentioned microorganisms for the three sample

Table 2: Minimum inhibitory concentration for each organism with regard to each sample

MIC (MINIMUM INHIBITORY CONCENTRATION)	**50µg/ml**	**5µg/ml**	**0.5 µg/ml**	**0.05µg/ml**	**0.005 µg/ml**	**0.0005 µg/ml**
S.Mutans(Group 1)						
sample 1	100%	100%	100%	100%	100%	100%
sample 2	68%	100%	100%	100%	100%	100%
sample 3	87%	5%	100%	100%	100%	100%
E.Faecalis(Group 2)						
sample 1	100%	100%	100%	100%	100%	100%
sample 2	77%	100%	100%	100%	100%	100%
sample 3	81%	100%	100%	100%	100%	100%
C.Albicans(Group 3)						
sample 1	100%	100%	100%	100%	100%	100%
sample 2	69%	64%	40%	100%	100%	100%
sample 3	69%	44%	20%	100%	100%	100%

DISCUSSION

Root canal treatment aims to remove bacteria and infected tissue from within the tooth. While mechanical instrumentation and chemical irrigation are essential parts of this process, they don't always reach every corner of the root canal system.

Several researchers have explored the use of alternative natural and synthetic medicaments for this purpose. For instance, Khalid et al. demonstrated that garlic extract combined with chitosan showed enhanced antimicrobial effects against *Enterococcus faecalis*, especially when used with MTA Fillapex sealer [6]. Similarly, Srinidhi et al. used chitosan as a carrier for antibiotic pastes and found results that support our own findings [7].

Calcium hydroxide, widely used material in endodontics, works by releasing hydroxyl (OH^-) ions that raise the pH and destroy bacterial cells [8]. However, this strong alkalinity can also affect dentin structure by reducing the strength of collagen-hydroxyapatite bonds, which could weaken the tooth over time [9,16]. Combining calcium

hydroxide with carriers like chitosan may help balance its effects—enhancing antimicrobial activity while minimizing damage.

Chitosan, derived from natural sources like crustacean shells, has gained attention for its antibacterial properties and biocompatibility. It's stable in acidic environments and degrades naturally over time, making it a good fit for medical applications [10]. Interestingly, the effectiveness of chitosan can vary depending on its molecular weight and structure—shorter polymer chains are said to offer better diffusion and activity [11–15].

In our study, the combination of 2% chitosan, dry ginger powder, and calcium hydroxide showed the most promising results. This trio significantly outperformed other groups in inhibiting the growth of *E. faecalis*, *S. mutans*, and *C. albicans*, both in terms of larger inhibition zones and lower MIC values. Ginger's well-documented antimicrobial components—such as gingerol and shogaol—may have added to the overall effect. The formulation appears to enhance the release and performance of calcium hydroxide, offering a synergistic benefit that could be clinically valuable. Hence, while the data support possible clinical value, translation into practice requires further validation through biofilm studies, dentin penetration models, and animal or clinical trials.

LIMITATIONS AND FUTURE DIRECTIONS

First, this was an in vitro study, and lab conditions cannot fully replicate the complexity of the human mouth. Factors like immune response, presence of saliva, and tissue interaction weren't simulated. Second, our sample size was relatively small, and the results may not generalize to broader populations. Third, chitosan's performance can vary depending on its source and molecular properties, which can introduce variability.

Additionally, we were unable to determine MIC values for *P. gingivalis* due to its strict anaerobic nature. Although we did measure its inhibition zone, this limits our understanding of how well the medicaments perform against this particular organism.

CONCLUSION

Within the limitations of this in vitro study, the combination of 2% chitosan, dry ginger powder, and calcium hydroxide exhibited the strongest antimicrobial activity against tested organisms. Chitosan alone showed minimal inhibition under the conditions employed, suggesting its role may be more effective as a carrier or synergistic agent. These results are preliminary and should be interpreted cautiously. Further research using dentin block models, biofilm assays, and clinical trials is required to confirm whether these findings can be translated into effective intracanal medicament strategies in endodontic practice.

CONFLICT OF INTEREST

To declare that they have no conflict of interests.

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