

Comparative Evaluation of Antibacterial Efficacy and Remineralization Potential of Acidulated Phosphate Fluoride Gel with Herbal Dental Gel Containing *Zingiber officinale*, *Salvadora persica*, and *Cinnamomum zeylanicum*: An *In Vitro* Study

Neha Kohli¹, Shivayogi M Hugar², Seema Hallikerimath³, Niraj Gokhale⁴, Krishna Kadam⁵, Sanjana P Soneta⁶

ABSTRACT

Aim: The aim of the study is to evaluate and compare the antibacterial efficacy and remineralization potential of acidulated phosphate fluoride (APF) gel with herbal dental gel containing *Zingiber officinale*, *Salvadora persica*, and *Cinnamomum zeylanicum*.

Materials and methods: Ethanolic extracts of *Z. officinale*, *S. persica*, and *C. zeylanicum* were prepared. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these extracts were determined against *Streptococcus mutans* and *Lactobacillus acidophilus* using the resazurin method and agar plate streaking method, respectively. The herbal dental gel was formulated, and its cytotoxicity was evaluated using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The antibacterial susceptibility of APF gel and the herbal dental gel was assessed using the agar well diffusion method and time-kill assay. The remineralizing potential was evaluated using a stereomicroscope.

Results: Herbal dental gel showed better antibacterial efficacy as depicted by the zone of inhibition of 20 and 21 mm obtained against *S. mutans* and *L. acidophilus*, respectively, compared to 11.50 and 16.50 mm zone of inhibition obtained by APF gel. The herbal dental gel also showed better remineralization potential than APF gel after a time interval of 24 and 48 hours, as depicted with a statistically significant *p*-value of 0.0061 and 0.0219, respectively. In the cytotoxicity test, the fibroblasts showed 100% viability in the presence of both study groups.

Conclusion: The results of our study concluded that herbal dental gel is safe and nontoxic, having anticariogenic potential due to its good antibacterial action and remineralization potential as compared to conventional APF gel.

Keywords: Antibacterial, Dental caries, Fluoride, Herbal, Remineralization.

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INTRODUCTION

According to Global Burden of Disease, dental caries is the most common oral health condition affecting 60–90% of children.¹ In spite of the foremost progress and advances in the field of preventive dentistry, the ever-increasing rate of caries in children has become a matter of concern.

Fluoride has been termed as the gold standard as it is widely recognized for reducing the prevalence of dental caries. However, with progressive research and scientific evidence, it has been deduced that fluoride is a double-edged sword.² Prolonged excessive intake of fluoride has been associated with dental fluorosis. It is a degenerative and progressive disorder that can also affect other organs such as the bones, thyroid, kidney, liver, lungs, and brain.³ Prolonged excessive intake of fluoride during pregnancy has been associated with mental problems in the developing fetus.⁴ Moreover, even with the extensive use of fluoride in our day-to-day lives, dental caries still dominate the spectrum of oral health diseases. Hence, it is the need of the hour to search for an effective and safe alternative.

During the last few decades, an uprising interest has been observed in the study of herbal extracts and their use in the different gamut of healthcare. Herbal extracts have been widely used in dentistry as anti-inflammatory, antimicrobial, antiseptic, antioxidant, antifungals, antibacterials, antivirals, and analgesics.⁵

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One of the commonly used herbs is *Zingiber officinale*, commonly known as ginger. It is well known for its medicinal uses, such as antimicrobial, antioxidant, anti-inflammatory,

hepatoprotective, anti-tumorigenic, antihyperglycemic, anti-lipidemic, antiemetic, and antipyretic.⁶ Another commonly used herb is *Salvadora persica*, most commonly known as miswak. It has a wide array of healthcare effects, including antibacterial, antimycotic, analgesic, carminative, diuretic, and astringent effects. It has also been used as a dental care tool since ancient times due to the ability of its fibers to reach between teeth and the richness of its phytoconstituents.⁷ *Cinnamomum zeylanicum* is also one of the most commonly used herbs, and it has numerous properties such as antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and anticancer.⁸

According to the existing literature, the synergistic effect pertaining to these herbs hasn't been explored. Hence, we planned this study to evaluate the antibacterial efficacy and remineralization potential of *Z. officinale*, *S. persica*, and *C. zeylanicum* by combining them as herbal dental gel and comparing them with commercially available acidulated phosphate fluoride (APF) gel.

MATERIALS AND METHODS

Preparation of Ethanolic Extract

All the herbs used in the study were verified by Shri BMK Ayurveda Mahavidyalaya, Belagavi. They were ground into fine powder using a pulverizer. The powdered samples were extracted with ethanol (Antares Chemicals Pvt. Ltd., Mumbai) in the ratio of 1:10 (v/v) for 4 days by keeping them inside a rotary incubator. The supernatant collected was filtered into a clean and sterile dried conical flask. The yield obtained was stored in plastic containers at room temperature (Fig. 1).

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were assessed against microbial-type culture collection strains of *Streptococcus mutans* and *Lactobacillus acidophilus*. The inoculum of standard strains of organisms was prepared per the 0.5 McFarland standard. The resazurin method and agar plate streaking method were used to determine MIC and MBC, respectively. Microbiological media was prepared using brain heart infusion (BHI) broth (HiMedia Laboratories Pvt. Ltd., Mumbai). A working solution of test compounds was prepared by adding 100 μ L of ethanolic extracts into 1 mL of dimethyl sulfoxide (DMSO)

(MERCK Specialty Pvt. Ltd). The standard operating protocols of vertical laminar flow were followed. In 96 well culture plates, 100 μ L of the microbiological media was added in specified wells. A total of 100 μ L of the working solution was added in the first well and then serially diluted up to the 12th well. Only 10 μ L of inoculum of bacteria was added in each well except positive control, and plates were incubated for 24 hours at 37°C. A total of 0.015% of resazurin was freshly prepared, and 20 μ L of this solution was added to each well. The plates were then incubated for 4 hours at 37°C. Active bacterial cells reduce the nonfluorescent resazurin (blue) to the fluorescent resorufin (pink), and the concentration at which this color change occurred was taken as MIC (Fig. 2).

The 96 well plates used for MIC determination with bacterial growth were streaked on the agar plate using platinum inoculation loops. These agar plates were incubated at 37°C for 24 hours and then observed for bacterial growth. The MBC value was determined when there was no colony growth from the directly plated contents of the wells (Fig. 3).

Preparation of Herbal Dental Gel

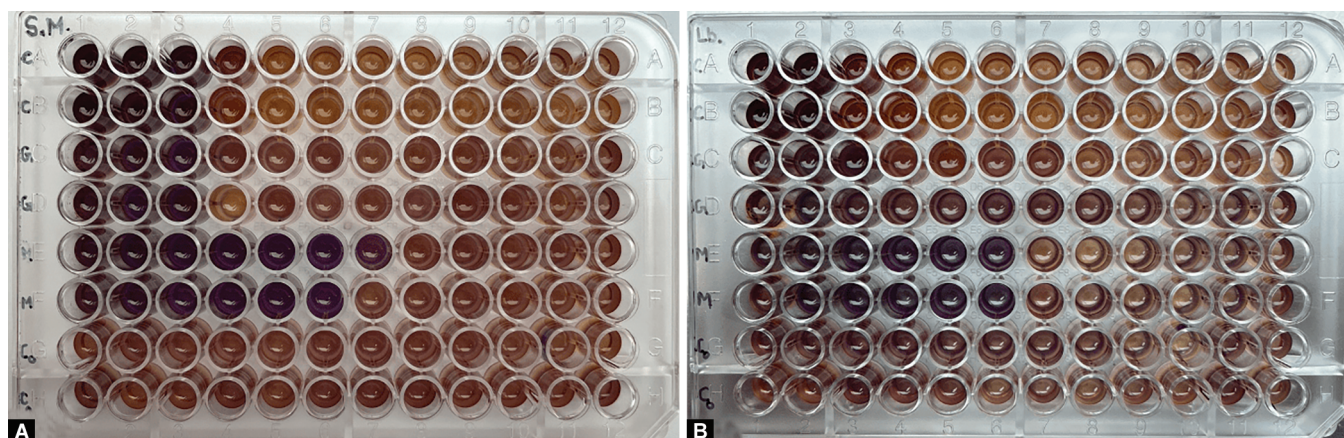
The herbal dental gel was prepared by mixing ethanolic extract of all herbs with following excipients—carbopol 940 (HiMedia Laboratories Pvt. Ltd., Mumbai, Maharashtra, India), glycerin (Molychem, Maharashtra, India), xylitol (HiMedia Laboratories Pvt. Ltd., Mumbai), sodium benzoate (SDFCL SD Fine Chemicals Ltd., Chennai, Tamil Nadu), methylparaben (SDFCL SD Fine Chemicals Ltd., Chennai, Tamil Nadu) and propylparaben (HiMedia Laboratories Pvt. Ltd., Mumbai). The pH of the gel was adjusted to 7.5 by adding triethanolamine (SDFCL SD Fine Chemicals Ltd., Chennai, Tamil Nadu), and it was transferred to the air-tight container kept at room temperature (Fig. 4).

Determination of Cytotoxicity Assay

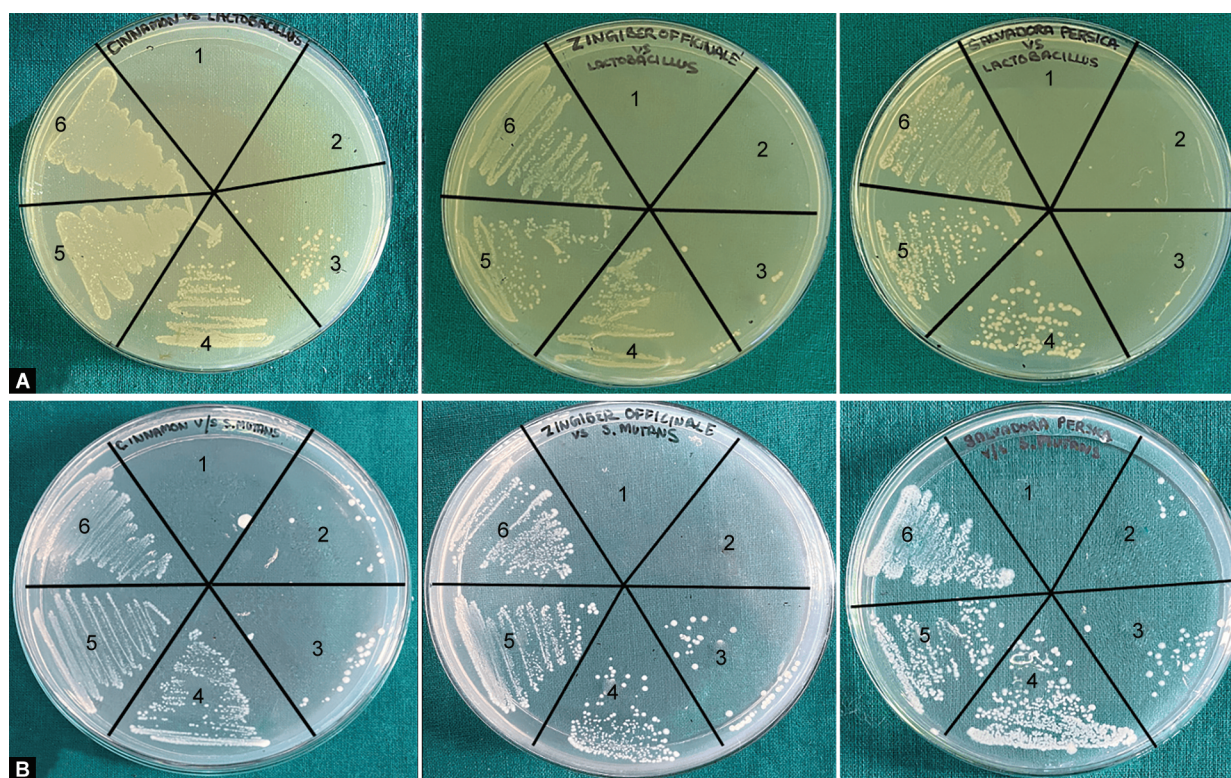
Cytotoxicity was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) (HiMedia Laboratories Pvt. Ltd., Mumbai) against L929 fibroblast (National Centre for Cell Science, Pune, Maharashtra). Detachment of cells from the flask was carried out by manually shaking them. Cells were centrifuged at 3500 RPM for 4 minutes. A small pellet was formed at the bottom, and the supernatant media was discarded. A total of 5 mL of fresh media was added and mixed thoroughly. Then, 50 μ L of 1×10^5 cells/mL cell suspension of the extract was seeded into



Figs 1A to C: Photograph showing the preparation of ethanolic extract of; (A) *Z. officinale*; (B) *S. persica*; (C) *C. zeylanicum*



Figs 2A and B: Photograph showing the determination of MIC of ethanolic extract of *Z. officinale*, *S. persica*, and *C. zeylanicum* against; (A) *S. mutans*; (B) *L. acidophilus*



Figs 3A and B: Photograph showing the determination of MBC of ethanolic extract of *Z. officinale*, *S. persica*, and *C. zeylanicum* against; (A) *S. mutans*; (B) *L. acidophilus*

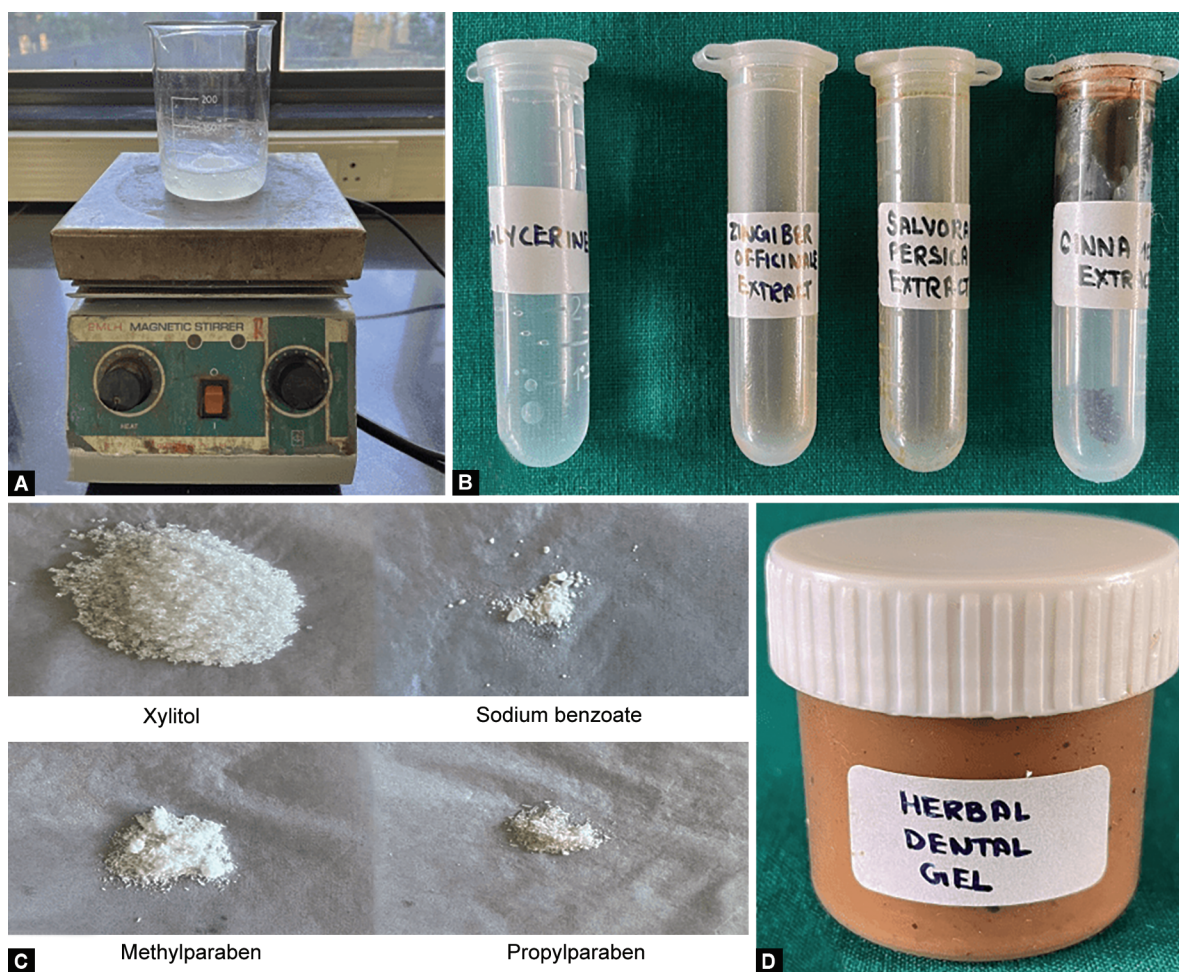
each well in a 96-well microtiter plate, and the final volume was made up to 150 μ L by adding Dulbecco modified eagle medium (Genetix Biotech Asia Pvt. Ltd. Media). Seeding of cells was done, followed by counting. They were incubated for 24 hours at 37°C in a CO₂ incubator. After 24 hours, 100 μ L of test compounds were added to the wells and incubated for 24 hours. Also, 20 μ L of MTT reagent was added to each well. The plate was kept for 4 hours of incubation. The supernatant was carefully removed without disturbing the precipitated formazan crystals, and 100 μ L of DMSO was added to dissolve the crystals formed. The optical density was measured at a wavelength of 492 nm using a microplate absorbance reader (Bio-Rad Laboratories India Pvt. Ltd., Haryana, India). The surviving fibroblasts were also observed under a microscope (Fig. 5).

Antibacterial Susceptibility Testing

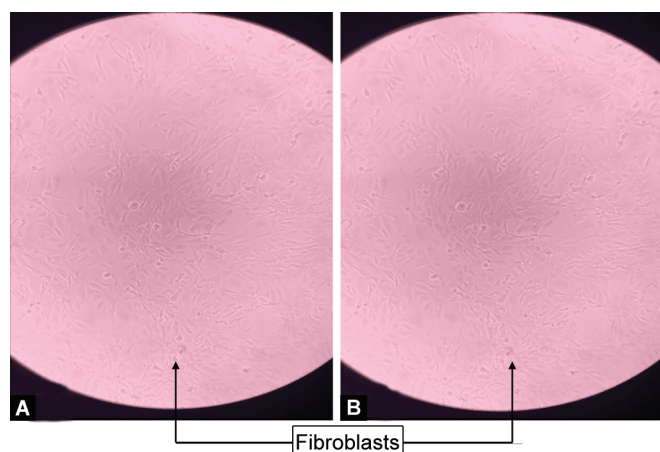
Antibacterial susceptibility for APF gel (Fluorovil Gel, Vishal Dentocare Pvt. Ltd., Ahmedabad) and Herbal dental gel was tested against *S. mutans* and *L. acidophilus* by agar well diffusion method and time-kill assay.

Procedure for Antimicrobial Susceptibility Using Agar Well Diffusion Method

Agar plates were inoculated with the standardized inoculum of the test microorganisms. Two wells with a diameter of 6–8 mm were punched aseptically, and the test compounds were introduced into the wells. The agar plates were then incubated for 24 hours. A zone of inhibition appeared, whose dimensions were recorded using a standardized scale (Fig. 6).

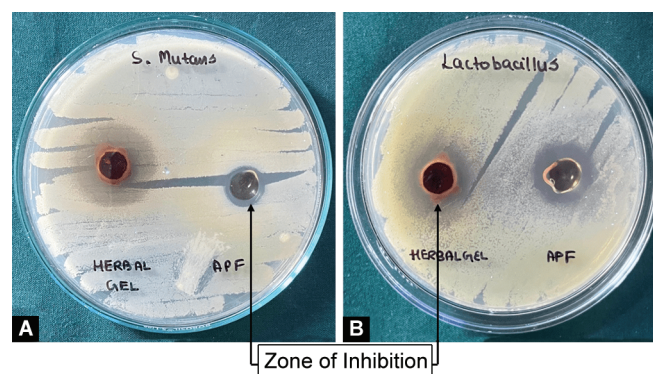


Figs 4A to D: Photograph showing steps of preparation of herbal dental gel



Figs 5A and B: Photograph showing a microscopic picture of the surviving fibroblasts with; (A) APF gel; (B) Herbal dental gel

Procedure for Antimicrobial Susceptibility Using Time-kill Assay
A solution of test compounds was prepared by adding 1 mL of BHI broth with 1 mL of APF gel and herbal dental gel, respectively. A total of 2 mL of aseptic broth was used as a negative control. To obtain the time-kill curve, the growth rate of bacterial strains was counted at different time intervals, that is, at 0, 2, 4, 6, 8, and 24 hours. The bacterial growth rates were determined from changes

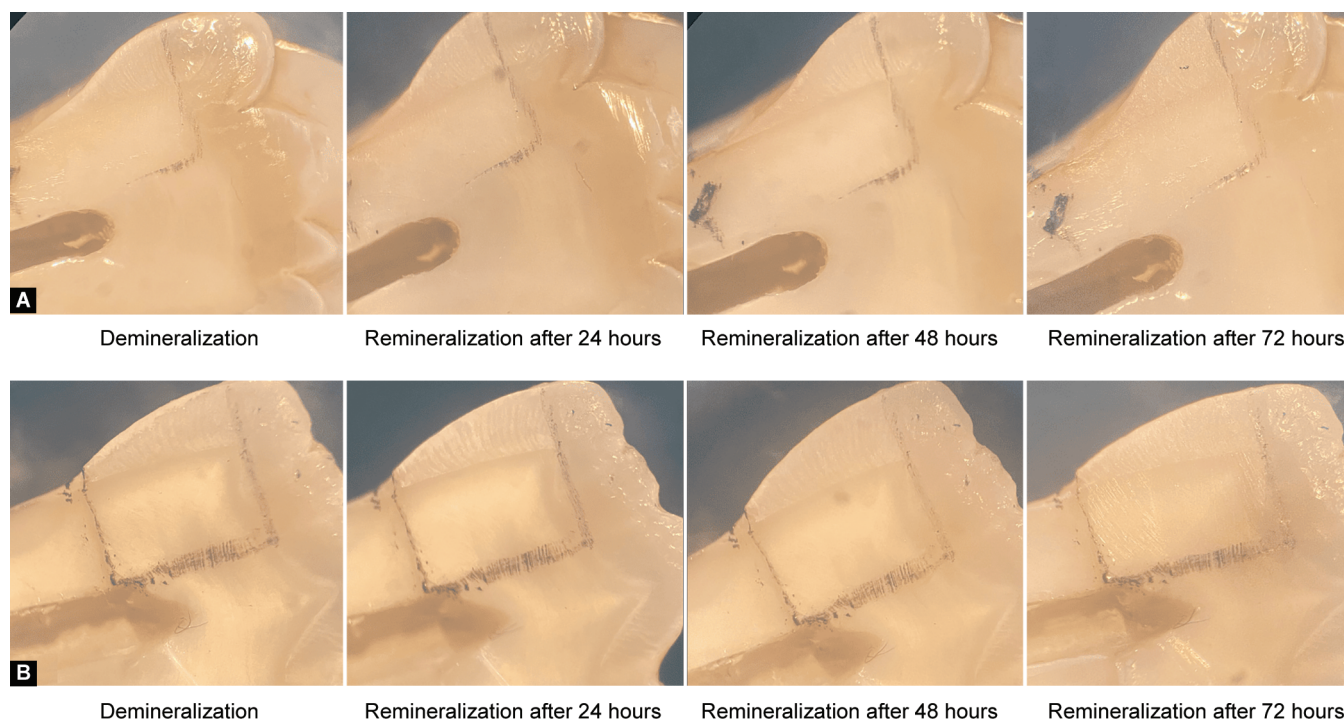


Figs 6A and B: Photograph showing zone of inhibition of APF gel and herbal dental gel against; (A) *S. mutans*; (B) *L. acidophilus*

in the density of viable bacteria whose readings were carried out using a spectrophotometer.

Evaluation of Remineralization

The estimated sample size for remineralization was calculated according to the standard sample size calculating formula, wherein a sample size of 30 was obtained for each group.⁹



Figs 7A and B: Photograph showing the evaluation of remineralization in; (A) APF gel; (B) Herbal dental gel group

Premolar teeth indicated for therapeutic extraction in children undergoing orthodontic treatment with intact crown structures were included in the study. Carious teeth, hypoplastic teeth, teeth with cracked areas, white spots, and discolored and fractured teeth were excluded from the study.

Teeth included in the study were stored in 10% formalin. They were dried and then longitudinally sectioned using a diamond disk in a buccolingual direction. The sectioned teeth were coated with acid-resistant nail varnish, leaving a rectangular window 4×3 mm wide for demineralization on the buccal surface. An artificial demineralizing lesion was created using 37% phosphoric acid (Ivoclar Vivadent, Schaan, Liechtenstein). These demineralized sections were then studied under a stereomicroscope (Labomed Inc., Los Angeles, United States of America) for evaluating lesion depth. Demineralized sections in group I were coated with APF gel and group II specimens were coated with herbal dental gel. After 24, 48, and 72 hours, the remineralized sections in both groups were observed under the stereomicroscope, and the lesional depth was measured using micrometer eyepiece as unit value (Fig. 7).

Statistical Analysis

The respective data was subjected to statistical analysis using IBM SPSS software (version 20.0, Bengaluru). Antibacterial tests were analyzed using the Chi-squared test to compare three groups and one-way analysis of variance (ANOVA) to compare intergroups. Cytotoxicity was statistically analyzed using Kruskal–Wallis one-way ANOVA. Statistical analysis for the evaluation of remineralization potential was done using the dependent *t*-test for comparison within groups and the independent *t*-test for comparison between groups.

RESULTS

Minimum inhibitory concentration of *Z. officinale* was found to be 11.25 ± 2.80 $\mu\text{L/mL}$ against *S. mutans* and 10.94 ± 9.38 $\mu\text{L/mL}$

against *L. acidophilus*. *S. persica* had a MIC of 1.56 ± 0.96 $\mu\text{L/mL}$ against *S. mutans* and 2.50 ± 0.86 $\mu\text{L/mL}$ against *L. acidophilus*. MIC of *C. zeylanicum* was found to be 11.25 ± 2.80 $\mu\text{g/mL}$ against *S. mutans* and 20.00 ± 6.85 $\mu\text{g/mL}$ against *L. acidophilus*.

Minimum bactericidal concentration of *Z. officinale* was found to be 27.50 ± 13.69 $\mu\text{L/mL}$ against *S. mutans* and 22.50 ± 5.59 $\mu\text{L/mL}$ against *L. acidophilus*. *S. persica* had an MBC of 13.75 ± 6.85 $\mu\text{L/mL}$ against *S. mutans* and 20.00 ± 6.85 $\mu\text{L/mL}$ against *L. acidophilus*. MBC of *C. zeylanicum* was found to be 27.50 ± 13.69 $\mu\text{g/mL}$ against *S. mutans* and 35.00 ± 13.69 $\mu\text{g/mL}$ against *L. acidophilus*.

On the evaluation of the cytotoxicity assay, fibroblasts showed 100% viability in the presence of both the study groups at 100% concentration. No statistically significant difference was found between the groups (Table 1).

The mean zone of inhibition obtained by APF gel against *S. mutans* was 11.50 mm, and herbal dental gel was 20.00 mm. The mean zone of inhibition obtained by APF gel against *L. acidophilus* was 16.50 mm, and herbal dental gel was 21.00 mm. These values indicate the superior antibacterial efficacy of herbal dental gel over APF gel (Table 2).

Figure 8 represents the time-kill assay scores of the APF gel and herbal dental gel against *S. mutans*. The continuous decrease in optical density values is depicted in the graph wherein it can be seen that APF gel showed an immediate reduction in *S. mutans* growth as evidenced by the reduction in the number of viable cells. Herbal dental gel presented an initial lag phase where bacteria were metabolically active but not dividing. After an interval of 7 hours, both groups showed equal efficacy in the reduction of *S. mutans* growth, as represented by the death phase characterized by an exponential decrease in the number of living cells. Both the test groups showed significant antibacterial activity against *S. mutans* as opposed to the control group, wherein exponential bacterial growth was observed.

Table 1: Table showing the mean of optical densities of surviving cells under APF gel and herbal dental gel while carrying out the cytotoxicity test using Kruskal–Wallis one-way ANOVA

	Absorption at 570 nm	Mean optical density	Standard deviation (SD)	Mean rank	Coefficient of variation	Percentage of viability	Interpretation
100% APF gel	0.291 0.489 0.481	0.42	0.11	5.67	26.66	100.82	No cell death
100% herbal dental gel	0.500 0.277 0.421	0.40	0.11	5.00	28.31	103.53	No cell death
Negative control	0.455 0.339 0.310	0.37	0.08	4.33	20.85	100.00	No cell death
H-value				0.3555			
p-value				0.8371			

Table 2: Table showing the antibacterial susceptibility using agar well diffusion test in APF gel and herbal dental gel against *S. mutans* and *L. acidophilus*

Groups	Round I	Round II	Mean
	Zone of inhibition against <i>S. mutans</i>		
APF gel	11	12	11.50
Herbal dental gel	21	19	20.00
Groups	Zone of inhibition against <i>L. acidophilus</i>		
APF gel	17	16	16.50
Herbal dental gel	19	23	21.00

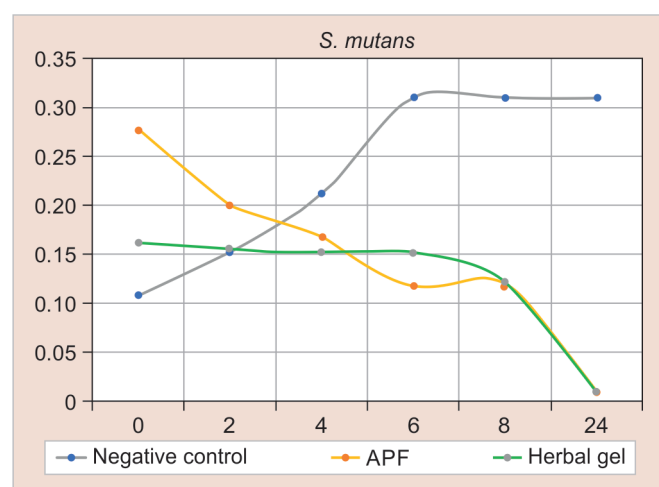
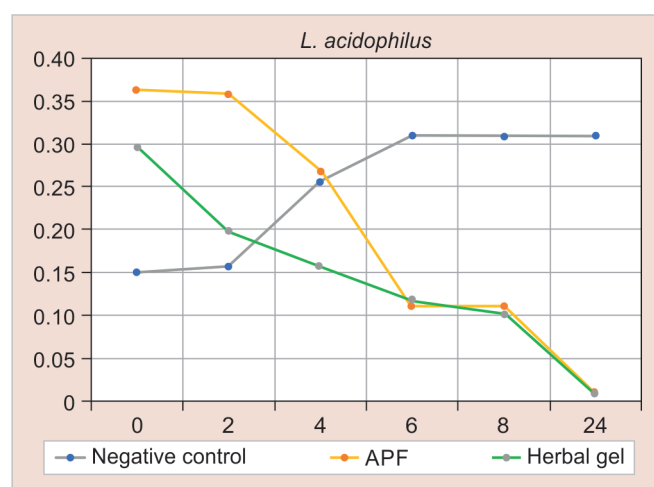
**Fig. 8:** Graphical representation of time-kill assay scores in APF gel and herbal dental gel against *S. mutans* at various time intervals

Figure 9 represents the time-kill assay scores of the two study groups against *L. acidophilus*. The continuous decrease in optical density values represents a significant antibacterial activity, as both study groups showed an immediate reduction in viable bacterial count. An exponential decrease in the number of living cells was observed after an interval of 6 hours in both groups, which represents the death phase of the bacteria. Both the study groups showed significant antibacterial activity against *L. acidophilus* as opposed to the control group, wherein exponential bacterial growth was observed.

Deminerization units before the application of test compounds were similar in the APF gel and herbal dental gel groups, indicating the standardization between both groups.

**Fig. 9:** Graphical representation of time-kill assay scores in APF gel and herbal dental gel against *L. acidophilus* at various time intervals

Mean remineralization after a time interval of 24 hours was 4.57 units in the APF gel group and 6.07 in the herbal dental gel group. These values indicate that remineralization potential at the end of 24 hours was higher in the herbal dental gel group, with a statistically significant difference as indicated by a p -value of 0.0061 ($p < 0.05$).

After 48 hours, the mean remineralization in the APF gel group was 6.83 units as compared to 8.23 units in the herbal dental gel group, indicating the superior remineralization potential of herbal dental gel. These values are also supported by a statistically significant p -value of 0.0219 ($p < 0.05$).

After the application of test compounds for 72 hours, mean remineralization in the APF gel group was 9.63 units as compared

Table 3: Table showing the comparison of remineralization rates in APF gel and herbal dental gel by independent t-test

Treatment times	APF gel		Herbal dental gel		t-value	p-value
	Mean	SD	Mean	SD		
24 hours after remineralization	4.57	1.59	6.07	2.41	-2.848	0.0061*
48 hours after remineralization	6.83	2.17	8.23	2.43	-2.354	0.0219*
72 hours after remineralization	9.63	3.17	11.00	2.64	-1.815	0.0746

*signifies statistically significant results with p -value < 0.05

to 11.00 units in the herbal dental gel. Although herbal dental gel showed more remineralizing potential than APF gel after an interval of 72 hours, there was no statistically significant difference. These results indicate that both the groups showed remineralization potential, but herbal dental gel showed superior remineralization potential over APF gel (Table 3).

DISCUSSION

In recent years, in the search for a novel agent with a good anticariogenic effect, there has been a tremendous increase in studies using natural plant extracts. However, based on the existing literature, there were no studies that explored the synergistic effect of these extracts not just as an antibacterial agent but also as a potential remineralizing agent.

The initial step is the extraction of the herb using a solvent in which its compounds are dissolvable. Ethanol was used as it contributes to the stability of the formulations and is considered one of the safest solvents for the extraction of herbal compounds.¹⁰ The ethanolic extracts were prepared by maceration due to their simplicity, as they do not require any complicated armamentarium.

Following this, the MIC and MBC of these extracts were determined to evaluate their individual potency against these microorganisms. In our study, *S. mutans* and *L. acidophilus* were used based on the observations made by various authors, who cite these microorganisms as the basic etiological agents of dental caries.^{11,12}

The resazurin method was used to determine the MIC, as it is known to have a high level of accuracy and reproducibility. The color change is easily appreciated by the naked eye, making this method highly feasible.¹³ For MBC, the agar plate streaking method was used because of its simplicity, low cost, and ease of interpretation of results.¹⁴ These tests were carried out in quintuplicates to ensure standardization of the results.

A vital step in the preparation of novel formulations is assessing their biocompatibility. In the present study, the gels were tested at 100% concentration, and it was found that the fibroblasts showed 100% viability in the presence of both the study groups, indicating their nontoxic effect.

A vital prerequisite for a potent anticariogenic agent is its antibacterial effect. The agar well diffusion method was employed in the study as it is easy to perform, does not require extensive armamentarium, and the results are easily interpreted.¹⁴ However, the agar well diffusion method only assessed the inhibition of bacterial growth, but not the bacterial death. In order to determine these effects, a time-kill assay was performed, which revealed a time-dependent antimicrobial activity. The findings obtained in the time-kill assay suggest that both APF gel and herbal dental gel have a similar time-dependent bactericidal action. However, as indicated by the results obtained in the agar well diffusion method, it can be implied that herbal dental gel has a superior antibacterial effect compared to APF gel in terms of the greater zones of inhibition obtained against both the test organisms.

This can be attributed to the constituents present in the herbal dental gel, which show a synergistic action in inhibiting the growth of the microorganisms. This can be deduced from the fact that the individual efficacy of these extracts was found to be less, as determined in the existing literature.

Z. officinale and *S. persica* have depicted an inhibitory zone of 8 mm against *S. mutans* in studies conducted by Giriraju and Yunus, and Balto et al.^{16,17} In a study conducted by Elgamily et al., *C. zeylanicum* showed an inhibitory zone of 14 mm against *S. mutans*.¹⁸ However, as per results obtained in our study, a combination of these extracts in the form of herbal dental gel has shown an inhibitory zone of 20 mm against *S. mutans*.

In a similar context, *Z. officinale* has shown an inhibitory zone ranging from 10 to 18 mm against *L. acidophilus*.¹⁹ In a study conducted by Siddeeqh et al., *S. persica* extract showed an inhibitory zone of 10.67 mm against *L. acidophilus*.²⁰ *C. zeylanicum* has depicted an inhibitory zone of 16 mm against *L. acidophilus* as per the results obtained in a study conducted by Elgamily et al.¹⁸ However, the herbal dental gel showed an inhibitory zone of 21 mm against *L. acidophilus*, which is superior to the individual antibacterial efficacy of these extracts.

These extracts have a specific mechanism of action by which they target the microorganisms. The antibacterial efficacy of *Z. officinale* can be attributed to the presence of gingerol and shogaol. They alter the permeability and fluidity of bacterial cell membranes. It is also known to have strong anti-adherence activity by inhibiting sucrose-dependent and sucrose-independent adherence. It also has an inhibitory effect on glucosyltransferase activity. It reduces the production of glycolytic acid by the bacteria and inhibits the F-ATPase activity, which gives rise to cytoplasmic acidity, resulting in disruption of the aciduricity potential of bacteria. It also leads to impairment of the functioning of enzymes involved in vital physiological processes like glycolysis, production of intracellular and extracellular polysaccharides, and cell persistence. Additionally, it downregulates the functioning of certain virulent genes like genes *relA*, *brpA*, *gtfC*, and *comDE*.²¹

S. persica also inhibits cell wall synthesis and has damaging effects on the cell membrane, resulting in an alteration of its permeability. This is mainly due to the presence of benzyl isothiocyanate, which penetrates through the membrane and interferes with the redox systems, hampering the ability of the bacteria to maintain its membrane potential. It also hampers the attachment of the bacteria to the surface.²² The presence of thiocyanate also acts as a substrate for lactoperoxidase, which generates hypothiocyanite, leading to cell death.²³ Additionally, it inhibits nucleic acid synthesis and protein synthesis, which causes interference in electron transportation, uptake of nutrients, and altered enzymatic activity. It can also result in the inhibition of oxygen uptake, leading to oxidative stress in bacteria and causing immediate toxicity and death.²⁰

The antibacterial activity of *C. zeylanicum* can be attributed to the presence of cinnamaldehyde, which is highly electronegative

and interferes with biological processes involving electron transfer. It reacts with nitrogen-containing components such as proteins and nucleic acids, resulting in the inhibition of growth of the microorganisms.²⁴ It also inhibits amino acid decarboxylase activity and acid production ability of the bacteria.²⁵ Additionally, it possesses antibiofilm activity due to the presence of glucosyltransferase adhesion-inhibition effect.²⁶

The results of the study also indicated the superior remineralization potential of herbal dental gel over APF gel. The reason that can be cited for the same is the presence of tannins in *Z. officinale*, which forms a layer over enamel due to their astringent effect and thus provides protection against demineralization.²⁷ *S. persica* is abundant in polysaccharides, which absorb directly onto the enamel and form protective layers over the surface. It is also abundant in chlorides and fluorides, which are important elements that remineralize the enamel structure.²⁸ It also produces an increase in calcium concentration in saliva, which induces remineralization.²⁹ The remineralization capability of *C. zeylanicum* is due to its rich calcium and phosphorus content, which are incorporated on the surface and decrease its porosity, eventually resulting in increased enamel microhardness.³⁰

Due to the promising results achieved in this study, we advocate the use of herbal dental gel for the prevention of caries in children. In addition to its efficacy as an anticariogenic agent, the herbal dental gel has also proven to be a safe formulation. However, as the results obtained in our study were *in vitro* analysis, we look forward to using this formulation in clinical scenarios by conducting clinical trials with a substantial sample size. We would also like to extend the use of this formulation to other delivery systems such as mouth rinses, dentifrices, or lozenges.

CONCLUSION

The results of our study conclude that herbal dental gel is safe and nontoxic, having anticariogenic potential due to its good antibacterial action and remineralization potential as compared to conventional APF gel.

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