

Long-Term Efficacy of Botanical Solutions in Maintaining Oral Health during Orthodontic Treatment: A Randomized Controlled Trial

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ABSTRACT

Introduction: Orthodontic appliances create plaque-retentive areas that compromise oral hygiene and increase the risk of dental caries and gingival inflammation, despite conventional preventive measures. Adjunctive antimicrobial strategies are essential to enhance plaque control and maintain optimal oral health during orthodontic treatment.

Aim: To evaluate the efficacy of probiotic, tea tree, curcumin, and sesame oil mouthwashes in modulating oral microbial flora, particularly *Streptococcus mutans*, among orthodontically managed patients.

Materials and Methods: This randomized controlled trial included 36 orthodontically treated patients divided into four groups, each assigned to a specific mouthwash: probiotic, tea tree, curcumin, and sesame oil. Saliva samples were collected at baseline (0 day), 30 days, and 60 days post-treatment initiation. Real-time PCR quantified *Streptococcus mutans* levels, while statistical analyses, including ANOVA and Tukey's HSD test, assessed changes in bacterial counts and compared the efficacy of the mouthwashes.

Results: Multivariate analysis suggested a trend towards significance in the effect of mouthwashes on oral microbial flora ($p \approx 0.062$), though individual statistical results did not meet conventional thresholds for significance ($p > 0.091$). Tukey's HSD test revealed no significant differences between the interventions ($p > 0.05$). Similarly, ANOVA showed no significant variation in bacterial counts among the groups at baseline (Day 0), 1 month (Day 30), and 2 months (Day 60) ($p > 0.05$). Despite the lack of statistical significance, the comparable performance of all mouthwashes suggests their potential utility in maintaining oral microbial balance during orthodontic treatment.

Conclusion: Probiotic, tea tree, curcumin, and sesame oil mouthwashes showed equivalent efficacy in modulating oral microbial flora in orthodontic patients. These results underscore the need for further research to explore their broader benefits and optimize their clinical application for enhanced oral health during orthodontic care.

Keywords: Curcumin mouthwash, dental caries, oral microflora, orthodontics, Probiotic mouthwash, sesame oil, tea tree mouthwash

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INTRODUCTION

Orthodontic treatment poses unique challenges in maintaining optimal oral health due to the presence of fixed or removable appliances, which create environments conducive to microbial plaque accumulation¹. These appliances, including brackets, wires, and bands, hinders effective oral hygiene practices and increase the risk of

dental caries, gingival inflammation, and periodontal issues². Traditional methods like brushing and flossing, while essential, often prove insufficient in reaching areas obscured by orthodontic appliances. Consequently, supportive and preventive care plays a pivotal role in mitigating the risks associated with orthodontic therapy³.

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Preventive strategies, such as the application of fluoride-rich and chlorhexidine varnishes, strengthen dental enamel and inhibit the growth of cariogenic bacteria, effectively reducing the incidence of dental caries⁴. Sealing dental grooves further prevents debris accumulation, while minimizing plaque-retaining items and dietary modifications aid in maintaining oral hygiene⁵. Despite these measures, orthodontic patients continue to face oral health challenges, highlighting the need for adjunctive solutions.

In recent years, alternative oral care products have garnered attention for their potential to complement traditional methods. Probiotic mouthwashes promote a healthy oral microbial balance by modulating the flora and reducing pathogenic risks^{6,7}. Tea tree oil is known for its antimicrobial properties against oral pathogens, while curcumin offers anti-inflammatory and antimicrobial benefits, supporting overall oral health. Similarly, sesame oil has demonstrated efficacy in reducing plaque accumulation and gingival inflammation⁸. These botanical and natural agents provide a promising approach to maintaining oral health during orthodontic treatment, yet their efficacy in orthodontic patients remains underexplored.

A comprehensive approach to oral health maintenance during orthodontic treatment must also consider patient compliance and comfort. Many orthodontic patients struggle to adhere to complex oral hygiene regimens, which underscores the importance of simple, effective, and accessible adjunctive products⁹. Botanical mouthwashes offer a natural, user-friendly alternative with minimal side effects, potentially enhancing patient compliance. By targeting oral microbial flora, these agents may not only reduce plaque and inflammation but also improve the overall treatment experience^{10,11}. Evaluating their effectiveness against conventional methods provides a pathway to refining oral hygiene practices in orthodontic care.

This study aims to evaluate the impact of probiotic, tea tree, curcumin, and sesame oil mouthwashes on the oral microbial flora of orthodontic patients. Using a randomized controlled trial design and advanced microbiological techniques, the study seeks to compare their efficacy and provide evidence-based recommendations for adjunctive oral hygiene strategies in orthodontic practice.

MATERIALS AND METHODS

Study Design and Period:

This study was designed as a randomized controlled trial conducted at the Department of Orthodontics, Thai Moogambigai Dental College, Chennai, over the period of one year from January 2023 to December 2023. The primary aim was to evaluate the effects of different mouthwash regimens on *Streptococcus mutans* levels in saliva among orthodontic patients treated with conventional metal brackets (Ormco™, 0.22 slot). Ethical approval was obtained from the Institutional Review

Board (IRB), and the procedures followed were in accordance with the ethical standards of the ethical committee on human experimentation (IRB) and with the Helsinki Declaration of 1975 that was revised in 2013. A written informed consent was collected from the parents of all participants prior to enrolment in the study.

Study Population and Selection Criteria:

The study included 36 patients undergoing orthodontic treatment who met the inclusion criteria. Patients were between the ages of 12 and 18 years and were treated with fixed orthodontic appliances using conventional metal brackets (Ormco™, 0.22 slot). Eligible participants were in good general health and exhibited no systemic conditions or medication use that could influence oral microbiota. Additionally, patients with no prior use of probiotics or antimicrobial mouthwashes in the preceding three months and those with good oral hygiene compliance were selected. Exclusion criteria included the presence of active periodontal disease, carious lesions, or unwillingness to participate in the study.

Group Allocation and Interventions:

Participants were randomly assigned to one of four intervention groups through a concealed allocation process to ensure blinding (Table 1). Each group was subjected to a specific mouthwash regimen over a one-month intervention period. Group A was administered a probiotic mouthwash, Group B used a tea tree oil mouthwash, Group C was given a curcumin-based mouthwash, and Group D utilized a sesame oil mouthwash. The intervention began on Day 30 of the study and continued until Day 60. Participants were instructed to use the mouthwash twice daily. Standardized toothbrushes and toothpaste (Colgate®) were provided to all participants to minimize variability. Adherence to the protocol was monitored, and any adverse effects were promptly reported.

Saliva Sample Collection:

Saliva samples were collected at three time points: baseline (Day 0), Day 30, and Day 60. At baseline, saliva was obtained prior to initiating orthodontic treatment. On Day 30, samples were collected after one month of orthodontic treatment without the use of mouthwash. Finally, on Day 60, saliva was collected following a one-month intervention with the assigned mouthwash. Saliva collection was conducted using paraffin wax pellets to stimulate salivary flow. Participants expectorated saliva into sterile 50 mL conical centrifuge tubes, which were transported to a -20°C storage facility within one hour of collection. Participants were advised to avoid consuming food or beverages for 30 minutes after using the mouthwash to maximize its efficacy.

DNA Extraction:

Genomic DNA was extracted from *Streptococcus mutans* isolates using the XpressDNA™ Bacteria Kit (MagGenome Technologies). During DNA extraction, overnight cultures of *S. mutans* UA159 were grown anaerobically at 37°C in Todd Hewitt broth until they

reached the late logarithmic growth phase. The bacterial cultures were centrifuged, and the resulting pellet was resuspended in phosphate-buffered saline. The optical density (OD) of the bacterial suspension was adjusted to 1.0, equivalent to 10^9 cells/mL, using a Bio-Rad™ SmartSpec Plus spectrophotometer. DNA extraction followed the kit's protocol, which included steps for lysis, binding, washing, and elution. The resulting DNA was of high quality and suitable for downstream applications such as quantitative real-time PCR.

Quantification of *S. mutans* using Real-Time PCR:

Quantitative real-time PCR (qPCR) was performed to quantify *S. mutans* DNA levels in saliva samples. Species-specific primers were designed to amplify *S. mutans*-specific sequences with high specificity. Serial tenfold dilutions of *S. mutans* DNA were prepared to generate standard curves, which were used to quantify the bacterial DNA based on cycle threshold (Cq) values. The qPCR reaction utilized the SYBR Green method, and primer specificity was validated against DNA from ten other bacterial species commonly found in the oral cavity. These standard curves allowed for the accurate determination of *S. mutans* DNA copy numbers in the samples.

Statistical Analysis:

All statistical analyses were conducted using SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize baseline characteristics. Repeated measures analysis of variance (ANOVA) was performed to assess changes in *S. mutans* levels across the different time points and intervention groups. Post hoc tests were conducted to determine intergroup differences. Statistical significance was set at $p < 0.05$.

RESULTS

Descriptive Statistics:

The study assessed *Streptococcus mutans* levels in saliva among orthodontic patients at three time points: baseline (Day 0), after one month of treatment without mouthwash (Day 30), and following one month of intervention with mouthwash (Day 60). The bacterial count was initially low at baseline across all groups, averaging 0.844 copies/mL ($p = 0.48$) (Figure 1). On Day 30, prior to mouthwash intervention, the bacterial count significantly increased to 1.898 copies/mL, likely due to the influence of orthodontic treatment on oral hygiene. After mouthwash intervention (Day 60), bacterial levels decreased to an average of 0.621 copies/mL across all groups, returning to levels comparable to baseline. (Figure 2)

ANOVA and Multivariate Analysis:

A repeated measures ANOVA was conducted to evaluate intra- and intergroup differences in *S. mutans* levels across the three time points. Multivariate tests, including Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root, revealed no statistically significant effect of mouthwash type on bacterial counts (Table 2). The p -value for the overall model was 0.062, indicating a trend toward significance but falling short of conventional thresholds. Further, all statistical measures yielded p -values above

0.091, demonstrating no notable differences between the effects of the different mouthwashes tested (Figure 3)

Post Hoc Analysis:

Multiple comparisons using Tukey's HSD test were performed to assess pairwise differences between interventions. The mean differences between mouthwashes (e.g., curcumin versus probiotic: -42832.66 , curcumin versus sesame oil: -7051.36 , curcumin versus tea tree: -4778.42) were not statistically significant ($p > 0.05$). Similar results were observed in other intergroup comparisons, indicating comparable effectiveness of the mouthwashes in reducing *S. mutans* levels (Table 3). The results indicate that orthodontic treatment may temporarily increase *S. mutans* levels, but the use of mouthwashes effectively mitigates this increase. However, no statistically significant differences were observed in the efficacy of the four tested mouthwashes—probiotic, curcumin, sesame oil, and tea tree oil. This suggests that all interventions performed similarly in reducing bacterial counts among orthodontic patients, emphasizing their potential utility in maintaining oral hygiene during orthodontic treatment.

DISCUSSION

The present study evaluated the effectiveness of probiotic, tea tree oil, curcumin, and sesame oil mouthwashes in orthodontic patients by assessing their impact on *Streptococcus mutans* levels. Orthodontic treatment often increases the risk of oral bacterial proliferation due to the creation of microbial niches around appliances. This study explored alternative oral care products to determine their comparative efficacy in mitigating this risk.

The findings indicated a trend toward significance in the impact of mouthwashes on *S. mutans* levels based on multivariate analysis, though pairwise comparisons using Tukey's HSD test revealed no statistically significant differences among the interventions. This suggests that all tested mouthwashes demonstrated similar efficacy in reducing bacterial counts. These results align with previous studies highlighting the potential of probiotic and herbal formulations in maintaining oral health during orthodontic treatment.

Probiotic mouthwashes have shown promise in reestablishing microbial balance by introducing beneficial bacteria. Deshmukh et al. demonstrated that probiotics effectively reduced *S. mutans* levels, thereby lowering caries risk¹¹. Tea tree oil, known for its antimicrobial properties, has also been reported to control oral pathogens responsible for dental diseases¹². Curcumin, a compound derived from turmeric, possesses both anti-inflammatory and antimicrobial effects, making it a promising agent for oral health¹³. Similarly, sesame oil has been shown to reduce plaque accumulation and gingival inflammation, as evidenced by the study conducted by Naseem et al¹⁴.

At baseline, the bacterial counts across all groups were relatively low, with no significant differences observed. However, following one month of orthodontic treatment without mouthwash use, a notable increase in bacterial

counts was observed, highlighting the impact of orthodontic appliances in creating niches conducive to bacterial proliferation. Upon introducing the mouthwashes, bacterial counts decreased, returning to baseline levels. These findings emphasize the comparable efficacy of the tested mouthwashes in mitigating bacterial proliferation during orthodontic treatment.

The tested alternative agents offer several advantages over conventional mouthwashes, such as chlorhexidine or alcohol-based formulations. Probiotic and herbal mouthwashes are generally associated with fewer side effects and lower risks of antimicrobial resistance. Additionally, their bioactive components, such as the anti-inflammatory properties of curcumin and the antimicrobial effects of tea tree oil, provide supplementary benefits for oral health.

These results are consistent with previous studies evaluating natural mouthwashes, such as green tea, neem, and aloe vera, which have demonstrated significant reductions in oral bacterial counts^{15, 16}. The findings of this study reinforce the potential of natural and probiotic formulations as effective adjuncts in maintaining oral hygiene during orthodontic treatment. However, the lack of statistically significant differences among the tested mouthwashes highlights the importance of considering individual patient factors, such as oral hygiene habits, microbial diversity, and diet, which may influence outcomes.

The use of advanced microbiological techniques, such as real-time PCR, is a key strength of this study, allowing for precise quantification of *S. mutans*. Furthermore, the inclusion of three distinct time points' baseline, one month (30days), and two months (60days) provided a robust framework for assessing bacterial dynamics. However, the study's relatively small sample size may have limited the statistical power to detect subtle differences among the interventions. Additionally, while *S. mutans* was the focus, other microbial species that may play a significant role in oral health were not considered.

Future studies should expand on these findings by incorporating larger sample sizes, assessing a broader range of microbial species, and exploring the long-term effects of these mouthwashes. Despite its limitations, this study underscores the potential of probiotic, tea tree oil, curcumin, and sesame oil mouthwashes as effective alternatives to conventional oral care products. These findings offer valuable insights into developing oral care regimens tailored to orthodontic patients.

CONCLUSION

The present study provides insights into the efficacy of probiotic mouthwash, tea tree mouthwash, curcumin mouthwash, and sesame oil in modulating the oral microbial flora of orthodontic patients. There is no statistical difference between the mouthwashes, which shows all mouthwashes were equal in efficiency as a probiotic mouthwash, which has also been supported by previous studies and is effective against *S. mutans*, as

shown in the ANOVA analysis. Further research is warranted to elucidate their effects on other oral health parameters and optimize their use in clinical practice. By incorporating adjunctive oral hygiene strategies, orthodontic practitioners can empower patients to maintain optimal oral health throughout their treatment journey, ultimately improving treatment outcomes and patient satisfaction.

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Declaration of Conflict of Interest: None

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Tables and Figures:

Table 1: Allocation of Sample in Each Group

Sample count after revealing the sample allocation	N
Curcumin Mouthwash	9
Tea tree Mouthwash	9
Sesame Oil Mouthwash	9
Probiotic Mouthwash	9
Total	36

Table 2: Multivariate tests of mouthwash effects and their interaction with real-time PCR data for *S. mutans* evaluation in orthodontic patients

Multivariate Factors	Test	Value	F	Hypothesis df	Error df	Sig.
factor1	Pillai's Trace	.169	3.061 ^b	2.000	30.000	.062
	Wilks' Lambda	.831	3.061 ^b	2.000	30.000	.062
	Hotelling's Trace	.204	3.061 ^b	2.000	30.000	.062
	Roy's Largest Root	.204	3.061 ^b	2.000	30.000	.062
factor1 * Real Time PCR Data compilation Table for <i>S. mutans</i> evaluation in orthodontic patients	Pillai's Trace	.186	1.060	6.000	62.000	.396
	Wilks' Lambda	.814	1.084 ^b	6.000	60.000	.383
	Hotelling's Trace	.228	1.104	6.000	58.000	.371
	Roy's Largest Root	.228	2.358 ^c	3.000	31.000	.091

^a- Exact Statistic, ^b- Adjusted for exact statistic ^c- Upper bound (Roy's Largest Root); factor1- Represents the time interval (baseline At 0, Day 30, Day 60))

Table 3: Tukey's HSD test in multiple comparisons analysis among interventions in Real-Time PCR Data for S. Mutans evaluation in orthodontic patients

Real-time PCR Data compilation for S.mutans evaluation in orthodontic patients to evaluate four interventions		Mean Difference (I-J)	Std. Error		95% Confidence Interval	
					Lower Bound	Upper Bound
Curcumin	Probiotic	-42832.66	25327.782	.345	-111574.08	25908.76
	Sesame Oil	-7051.36	26192.459	.993	-78139.58	64036.86
	Tea tree	-4778.42	25720.484	.998	-74585.66	65028.83
Probiotic	Curcumin	42832.66	25327.782	.345	-25908.76	111574.08
	Sesame Oil	35781.30	21106.485	.343	-21503.22	93065.82
	Tea tree	38054.24	20517.848	.268	-17632.67	93741.16
Sesame Oil	Curcumin	7051.36	26192.459	.993	-64036.86	78139.58
	Probiotic	-35781.30	21106.485	.343	-93065.82	21503.22
	Tea tree	2272.94	21576.155	1.000	-56286.30	60832.18
Tea tree	Curcumin	4778.42	25720.484	.998	-65028.83	74585.66
	Probiotic	-38054.24	20517.848	.268	-93741.16	17632.67
	Sesame Oil	-2272.94	21576.155	1.000	-60832.18	56286.30

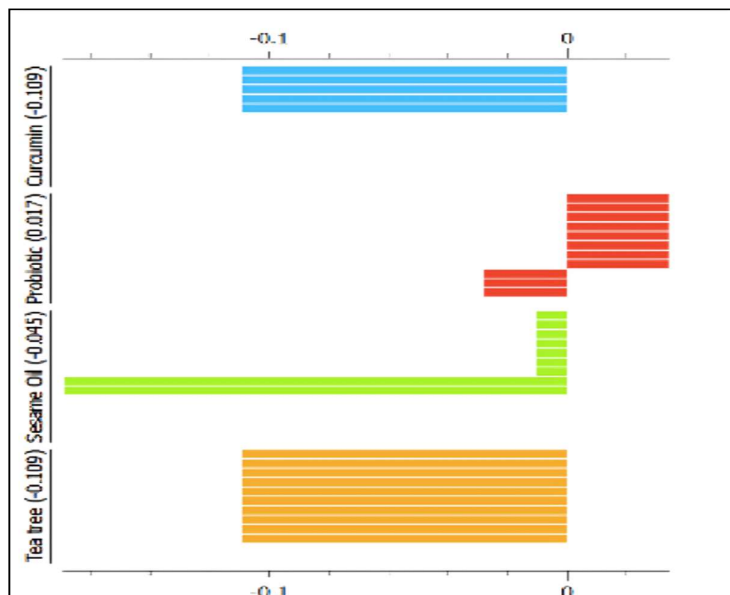


Figure 1: Silhouette graph showing the distribution of four types of mouthwash based on the compiled Rt-PCR 2delta CT values

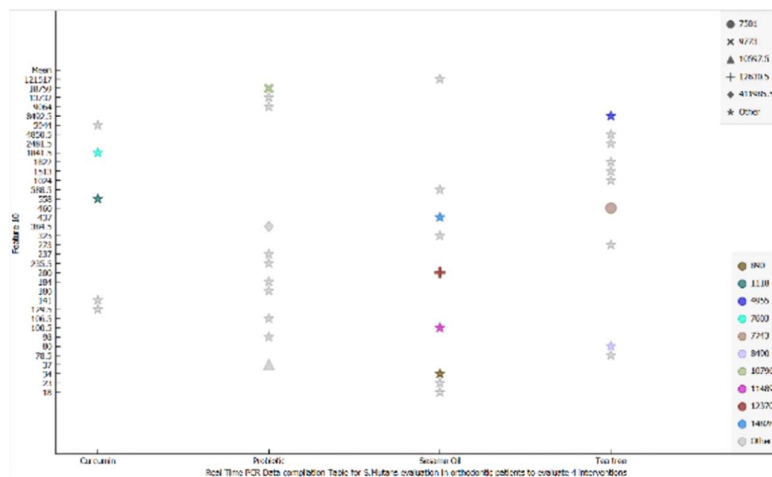


Figure 2: Scatter plot showing the distribution of four types of mouthwash based on the compiled Rt-PCR 2delta CT values

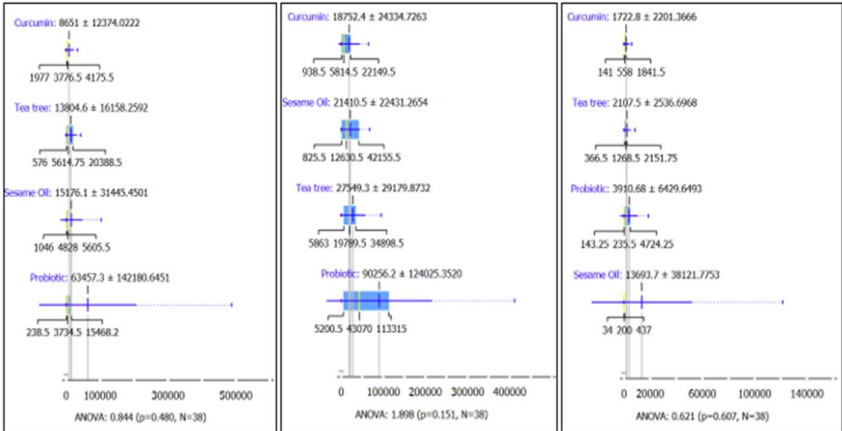


Figure 3: ANOVA results of the baseline period (Day 0), Day 30 and Day 60 Time period from left to right