Original Article

Efficacy of aloe vera and probiotic mouthwashes vs fluoride mouthwash on *Streptococcus mutans* in plaque around brackets of orthodontic patients: a randomized clinical trial

Nisha D S[a]; Biju Sebastian[b]; Rishad Kalappurakkal[c]; Richard Kirubakaran[d]

ABSTRACT

Objectives: To compare efficacy of aloe vera and probiotic mouthwashes vs fluoride mouthwash on *Streptococcus mutans* (*S. mutans*) in the plaque of orthodontic patients and to assess patient-reported outcomes and compliance.

Materials and Methods: This prospective randomized clinical trial included 90 patients aged 12–35 years and in permanent dentition, who were randomly allocated in a 1:1:1 ratio to three mouthwash groups: aloe vera, probiotic, or fluoride. Smartphone-based applications were used to improve patient compliance. The primary outcome was the change in *S. mutans* levels in plaque between two times: pre-intervention and after 30 days using real-time polymerase chain reaction (Q-PCR). Secondary outcomes were the evaluation of patient-reported outcomes and compliance.

Results: Mean differences between aloe vera vs probiotic: −0.53 (95% CI: −3.57 to 2.51), aloe vera vs fluoride: −1.99 (95% CI, −4.8 to 0.82), and probiotic vs fluoride: −1.46 (95% CI: −4.74 to 1.82) were not significant, $P = .467$. Intragroup comparison demonstrated a significant mean difference in all three groups of −0.67 (95% CI: −0.79 to −0.55), −1.27 (95% CI: −1.57 to −0.97), and −2.23 (95% CI: −2.44 to −2.00) respectively, $P < .001$. Adherence was above 95% in all groups. No significant differences in frequency of responses to patient-reported outcomes were found among groups.

Conclusions: No significant difference in efficacy among the three mouthwashes in reducing *S. mutans* level in plaque was found. Patient-reported assessments concerning burning sensation, taste, and tooth staining found no significant differences among mouthwashes. Smartphone-based applications can help improve patient compliance. (*Angle Orthod.* 2023;93:538–544.)

KEY WORDS: Probiotic; Aloe vera; Q-PCR; Plaque *Streptococcus mutans*; Patient compliance

INTRODUCTION

Enamel demineralization leading to white spot lesions (WSLs) has been a major iatrogenic esthetic concern in orthodontic practice despite recent advances in orthodontic materials and techniques.[1] In fixed orthodontic appliances, brackets and archwires act as retention niches, creating an ideal plaque accumulation and microbial multiplication environment. Among the causative pathogens, *Streptococcus mutans* (*S. mutans*) level in plaque strongly correlates with WSLs.[2]

Currently, the recommended best practice for orthodontic patients with fixed appliances is daily rinsing with fluoride mouthwash along with the use of toothpaste.[3–5] This has helped in retarding, but not wholly arresting, WSLs.[6] Also, over 50 years of fluoride treatment has led to fluoride-resistant *S. mutans* strains.[7] Hence there is a need for new antimicrobial mouthwashes active against *S. mutans*. Aloe vera mouthwash is a natural herbal alternative found to reduce plaque and gingival inflammation,[8] and it inhibits the growth of diverse oral microorganisms, including *S. mutans*.[9–12] It is also safe and well-tolerated with no or very minimal side effects.[8] Another potential product is probiotic mouthwash,

[a] Resident, Department of Orthodontics, Pushpagiri College of Dental Sciences, Kerala, India.
[b] Former Professor and Head, Department of Orthodontics, Pushpagiri College of Dental Sciences, Kerala, India.
[c] Research Director, Unibiosys Biotech Research Labs, Kochi, Kerala, India.
[d] Research Scientist, Centre for Biostatistics and Evidence-based Medicine, Vellore, India.
Corresponding author: Dr Biju Sebastian, Department of Orthodontics, Malabar Dental College and Research Centre, Edappal, Kerala 679578, India
(e-mail: drbijuseb00@gmail.com)

Accepted: February 2023. Submitted: August 2022.
Published Online: May 3, 2023

Table 1. Ingredients of Mouthwashes and Toothpaste

Product	Ingredients
Aloe Vera Mouthwash Dr Organic Aloe Vera Mouthwash (Dr Organic Ltd, United Kingdom)	Aloe barbadensis leaf juice, aqua, sorbitol, polysorbate 20, glycerin, Cetraria islandica (Icelandic moss) extract, Pyrus malus (apple) fruit extract, Citrus grandis (grapefruit) extract, Centella asiatica (Indian pennywort) extract, Mentha piperita (peppermint) oil, menthol, Sodium lauroyl sarcosinate, aroma, xylitol, Melaleuca alternifolia (tea tree) leaf oil, Arnica montana extract, sodium hydroxymethylglycinate, sodium benzoate, potassium sorbate, citric acid, CI 75810 (chlorophyll), Limonene, linalool
Probiotic mouthwash Bioven ingredients *Saccharomyces boulardii* (Biovencer Healthcare Pvt Ltd, India)	Lyophilized *Saccharomyces boulardii*, excipients
Amine fluoride mouthwash Amflor mouthwash 0.2% amine fluoride (Group Pharmaceuticals Ltd, India)	Amine fluoride 480 ppm, purified water, sorbitol, sodium saccharin, poloxamer, propylene glycol, sodium benzoate, hydrogenated castor oil
Toothpaste Generic toothpaste (Pushpagiri College of Pharmacy, India)	Dicalcium phosphate dihydrate (DCP-D), sodium lauryl sulphate, glycerin, gum tragacanth, water, sodium saccharine

and several probiotic agents are effective against *S. mutans*.[13–15]

To date, no study has evaluated the efficacy of these mouthwashes on *S. mutans* in orthodontic patients. Therefore, this randomized clinical trial (RCT) was undertaken to assess and compare the efficacy of aloe vera and probiotic mouthwashes to that of fluoride mouthwash on *S. mutans* in plaque surrounding brackets. Secondary objectives included evaluation of adverse effects of the mouthwashes with qualitative and sensory assessments. In addition, patient compliance with the assigned mouthwash use was also assessed.

MATERIALS AND METHODS

The prospective study was planned as a three-arm parallel-group, randomized (1:1:1), triple-blind, active control trial, and there were no alterations in methods after commencement. The trial was approved by the Institutional Ethics Committee (study reference number: L1/11/18 and registered at http://ctri.nic.in/Clinicaltrials/login.php (CTRI/2021/05/033630).

Consecutive patients who were to undergo orthodontic treatment with fixed appliances and stainless-steel brackets and who consented to the trial were enrolled at the postgraduate orthodontic clinic of the Pushpagiri College of Dental Sciences from May 2021 to July 2021. The patients were from a population that consumed nonfluoridated water. The recruited sample of 90 patients satisfied the following selection criteria: (a) age between 12 and 35 years and in permanent dentition with all anterior teeth present, (b) no systemic diseases that might interfere with the trial, (c) healthy periodontium, and (d) no previous orthodontic treatment. Participants were excluded in the presence of (a) composite restorations and prosthetic crowns on anterior teeth, (b) teeth with developmental anomalies or fluorosis, (c) need for anti-inflammatory or antibiotic medication, (d) pregnant women and smokers, and (e) history of frequent vomiting. Before recruitment, signed consent was obtained from each participant and the parents or legal guardians if the participants were adolescents.

Interventions

Before the start of fixed orthodontic treatment, all patients were provided an oral hygiene kit every month containing a toothbrush (Colgate Slim Soft Ortho Brush, Colgate-Palmolive Company, India), an interdental brush (Colgate Total Interdental Brush, Colgate-Palmolive), dental floss (Oral-B Essential Floss Unwaxed, Procter and Gamble, Cincinnati, Ohio), and a tube of nonfluoridated toothpaste. Oral hygiene instructions were given and professional prophylaxis was done including supragingival scaling and polishing with nonfluoride containing pumice. Participants were bonded with 0.022-inch × 0.028-inch slot MBT prescription conventional stainless-steel brackets (Victory series, 3M Unitek, Monrovia, Calif) in both arches using a nonfluoride primer and adhesive (Transbond XT, 3M Unitek). Excess adhesive flash was removed either with a scaler before curing or with a tungsten carbide bur after curing. All participants were instructed to follow the modified Bass technique of tooth brushing after applying 2 cm (approximately 1 g) toothpaste on the brush and to brush twice daily for 2 minutes, once in the morning and night with the provided nonfluoridated toothpaste (Table 1). Participants were advised to avoid fluoride-containing products, chewing gum, carbonated soft drinks, and acidic juices.

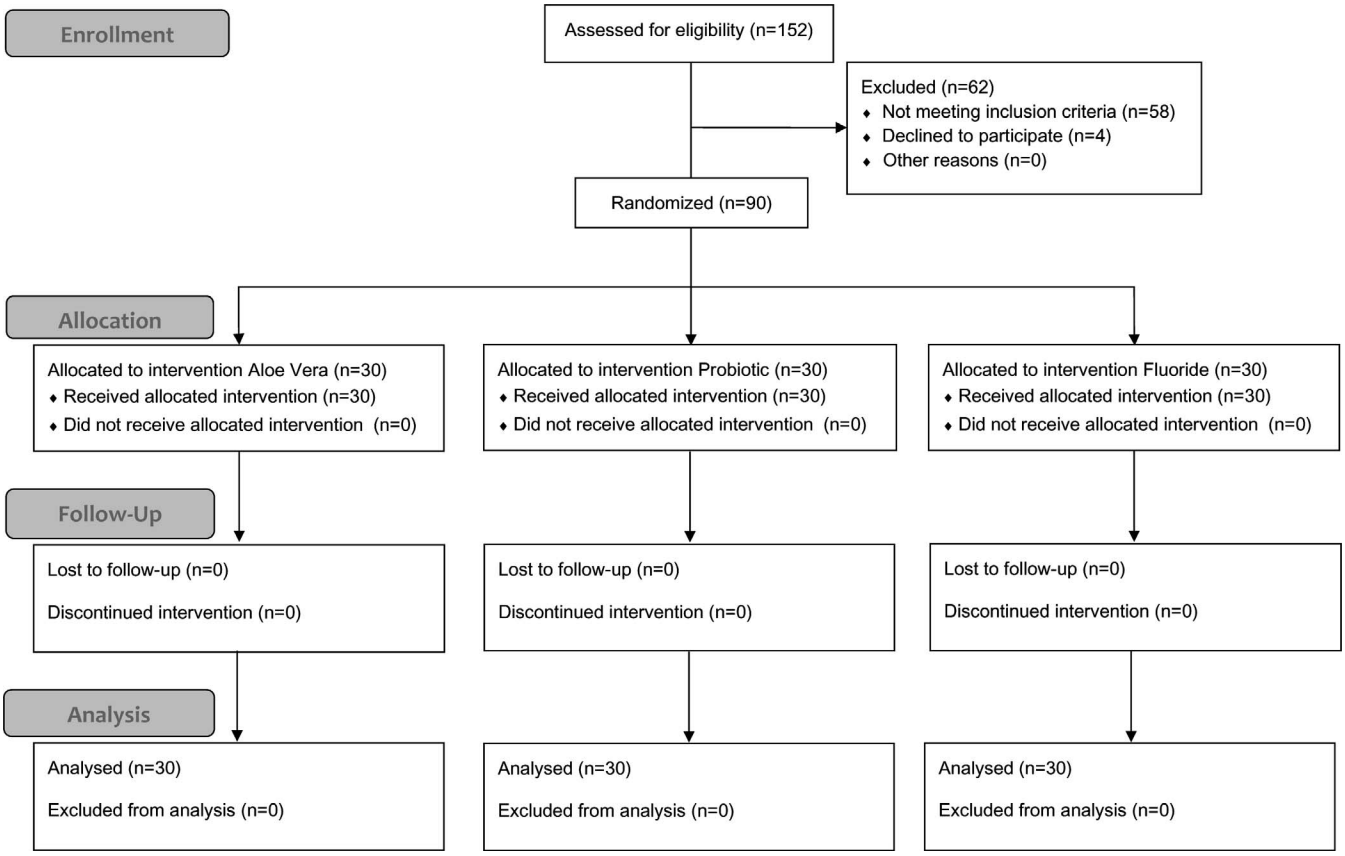

Figure 1. CONSORT diagram showing the flow of participant data during the trial.

At the scheduled appointment after 3 months of orthodontic treatment (baseline visit: T0), the archwires were removed for plaque specimen collection using the four-pass technique.[16] The specimens were obtained from around each upper lateral incisor bracket base by moving the tip of a sterilized dental scaler (8/9 Orban DE hoe scaler, Hu-Friedy, Chicago, Ill) along the bracket circumference.

Each participant was then randomly assigned to one of the three mouthwash groups: Group 1 (aloe vera), Group 2 (probiotic), or Group 3 (0.2% amine fluoride), where Groups 1 and 2 were the intervention groups, and Group 3 was the active control group. The details of the commercial mouthwash products and their ingredients are given in Table 1. For the next 30 days, all participants were directed after tooth brushing to rinse their teeth twice daily for 1 minute with the allotted mouthwash before expectorating and to follow the manufacturer's directions for use. They were instructed to avoid rinsing their mouth, drinking, or eating for at least 30 minutes after tooth brushing and mouthwash use. After 30 days of mouthwash use (postintervention visit: T1), the elastomeric modules and archwires were again removed for plaque specimen collection using the four-pass technique.

Patient Compliance

At T0, patients received a motivational video on oral hygiene maintenance and the importance of using mouthwashes to prevent WSLs on their smartphones in addition to verbal instructions. Participants were also encouraged to download similar smartphone-specific videos during their study period. A mobile app calendar-based system (https://calendar.google.com) was installed on their smartphones, programmed to send active reminder notifications to participants twice daily at the scheduled time of mouthwash rinsing. In addition, patients were enrolled in a WhatsApp-based anonymous chat room (all patients used a unique identification [ID]) moderated by the department practice manager. Patients were told not to share photographs or text messages that could disclose their real identity in the chat room. Upon completing the task of mouthwash rinsing, patients were directed to post a confirmatory message each time in the WhatsApp group. Two department auxiliary personnel were responsible for monitoring the responses and, if needed, telecommunicating with patients. The measured compliance was assessed by counting the number of instances a patient used the mouthwash

Table 2. Baseline Sample Characteristics of Participants[a]

Variable	Aloe Vera Mouthwash (n = 30)	Probiotic Mouthwash (n = 30)	Fluoride Mouthwash (n = 30)
Age, y, mean (SD)	19.53 (±5.72)	18.40 (±3.92)	18.40 (±3.81)
Sex, n (%)			
Female	15 (50)	15 (50)	14 (46.67)
Male	15 (50)	15 (50)	16 (53.33)
Incisor class, n (%)			
Class I	16 (53.33)	15 (50)	16 (53.33)
Class II	11 (36.67)	11 (36.67)	12 (40)
Class III	3 (10)	4 (13.33)	2 (6.67)

[a] SD indicates standard deviation.

according to the confirmatory messages posted in the WhatsApp-based anonymous chat room.

Qualitative and Sensory Assessment Questionnaire

At T1, a questionnaire was provided to each participant to quantify the taste, burning sensation, and tooth staining tendency of the mouthwashes on an ordinal scale assigned from 0 to 10, according to the method suggested by Nogueira et al.[17] The scores obtained were then categorized (No, Light, Moderate, Severe, Very severe) and distributed on a new scale for each outcome variable.[17]

Microbiologic Analysis

Q-PCR was performed using SYBR green for the relative detection of *S. mutans* in DNA isolated from oral plaque samples. The primers for the analysis were obtained from a previously published paper,[14] and the primers specifically targeted the conserved region of the glycosyltransferase gene (gtf B) of *S. mutans*. The assays underwent up to 40 cycles of amplification. The cycle threshold value (number of cycles) at which there was the genomic expression of gtf B was obtained in each plaque sample. The specificity in amplification of the gene gtf B was confirmed based on the annealing temperature of positive control, taken as 63°C. The cycle threshold (Ct) values of plaque samples before and after interventions in each subject were obtained.

Sample Size Calculation

A 1-month-long pilot study was conducted due to the lack of similar clinical studies in orthodontic patients. The reduction in the mean *S. mutans* level detected in this pilot study was used as reference to calculate the sample size (n = 27) in each arm with a power of 0.80, at the 0.05 significance level, and effect size of 0.84. Considering an anticipated 10% dropout rate, this was rounded up to 30 participants.

Randomization

Randomization was based on computer-generated pseudorandom code using random permuted non-stratified blocks of six, ensuring equal distribution in the three arms (R-software base version 4.1.0 for Windows). The block size was not disclosed, and each participant was given a unique ID. Allocation concealment was ensured by the centralized assignment protocol managed by the research unit supervisor who released the randomization code using sequentially numbered, sealed, opaque envelopes. The randomization list generation, allocation concealment, and implementation (enrollment/treatment assignment/intervention delivery) were independently performed by different individuals.

Blinding

The assigned mouthwash products were dispensed in identical opaque bottles and coded as 1, 2, or 3. Neither the clinician nor the participants received any information on the products. The clinician was not involved in any randomization procedures or product delivery. Hence the participant and clinician were blinded throughout the study. The assignment of the unique ID to each participant ensured the blinding of the laboratory investigators.

Statistical Analysis

All data were analyzed using SPSS software (version 25; IBM, Armonk, NY), and the results were considered statistically significant at $P < .05$. Kolmogorov-Smirnov test was used to assess the normality of data distribution. Descriptive statistics were shown as means and standard deviation for continuous variables, and frequencies and percentages for categorical variables. An analysis of the patient demographic characteristics and type of malocclusion was performed. Considering that the variables fulfilled a normal distribution, one-way analysis of variance (ANOVA) was used to compare the cycle threshold values among the mouthwashes (intergroup compar-

Table 3. Intergroup and Intragroup Comparison of Cycle Threshold Values

	Aloe Vera Mouthwash (n = 30)			Probiotic Mouthwash (n = 30)		
	Cycle Threshold Value (Mean ± SD)	Change From Baseline		Cycle Threshold Value (Mean ± SD)	Change From Baseline	
		Absolute Change Mean (95% CI)	*P* Value**		Absolute Change Mean (95% CI)	*P* Value**
Baseline	30.90 ± 4.81	−0.67 (−0.79 to −0.55)	<.001	29.76 ± 6.50	−1.27 (−1.57 to −0.97)	<.001
Postintervention	31.57 ± 4.89			31.04 ± 6.73		

* One-way analysis of variance.
** Paired *t*-test.

isons) and paired *t*-test to evaluate the effect within a mouthwash group on cycle threshold values (intragroup comparisons).

The Fisher exact test was performed to compare the responses of patient-reported outcomes among the mouthwashes. Self-reported adherence percentage was calculated to evaluate and compare the patient compliance with mouthwash rinsing.

RESULTS

All ninety patients completed the trial (Consolidated Standards of Reporting Trials [CONSORT] flow diagram; Figure 1). The baseline patient characteristics regarding age, sex, and incisor classification were similar in all groups (Table 2).

Primary Outcome

The mean Ct value differences between mouthwash groups were as follows: aloe vera vs probiotic: –0.53 (95% CI: –3.57 to 2.51), aloe vera vs fluoride: –1.99 (95% CI: –4.8 to 0.82), and probiotic vs fluoride: –1.46 (95% CI: –4.74 to 1.82). The intergroup comparison from one-way ANOVA presented no statistically significant difference in the postintervention Ct values, $P = .467$ (Table 3).

The Ct values before and after interventions of aloe vera, probiotic, and fluoride mouthwash groups had a mean difference value of –0.67 (95% CI: –0.79 to –0.55), –1.27 (95% CI: –1.57 to –0.97), and –2.23 (95% CI: –2.44 to –2.00), respectively, with paired *t*-test demonstrating a $P < .001$ (Table 3).

Secondary Outcomes

The self-reported adherence percentage was calculated as mean (minimum to maximum value) for each mouthwash group (n = 30) and were: 0.954% (0.917%–100%), 0.961% (0.867%–100%) and 0.959% (0.917%–100%) for aloe vera, probiotic, and fluoride mouthwash groups, respectively. Above 95% adherence meant excellent patient compliance in all three groups (Figure 2).

As for patient-reported outcomes (Table 4), no significant differences were found between the groups regarding the frequency of responses to each outcome: burning sensation ($P = .512$), taste ($P = .393$), and tooth staining ($P = .249$).

DISCUSSION

The long-term prescription of antimicrobial chemotherapeutic agents like fluoride or chlorhexidine for orthodontic cases can cause several adverse effects, including discoloration of teeth and drug resistance.[7,13,18,19] Therefore, naturally-derived mouthwashes such as aloe vera or probiotic, which could have an inhibitory effect on *S. mutans*,[9,11–15,20] may be a safer option.[8,21,22] Among the products, fluoride was included as active control since it is a proven[13,23,24] and widely used chemotherapeutic agent in many orthodontic oral care products such as toothpaste, mouthwashes, and gels. The association with amine enhances the antibacterial activity of fluoride.[24]

The present study on patients undergoing fixed orthodontic treatment demonstrated no significant difference in the efficacy among the three mouthwashes: fluoride, probiotic, and aloe vera in reducing *S. mutans* level in plaque. The within-group comparison of all three mouthwashes demonstrated their efficacy against *S. mutans*. No comparisons of these results

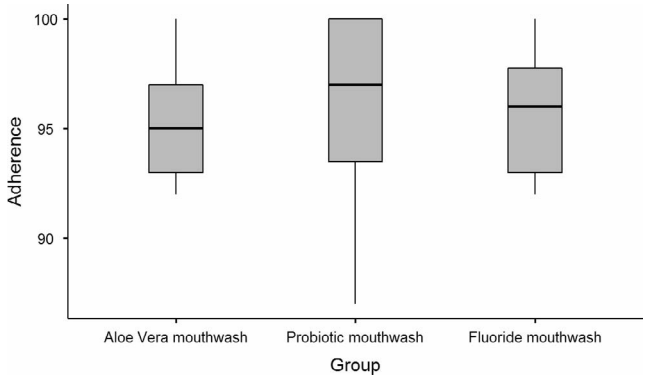

Figure 2. Box and whisker plot showing self-reported adherence % of mouthwash groups.

Table 3. Extended

Fluoride Mouthwash (n = 30)			Mean Difference Between Groups (95% CI)			
Cycle Threshold Value (Mean ± SD)	Change From Baseline: Absolute Change Mean (95% CI)	*P* Value**	Aloe Vera vs Probiotic	Aloe vera vs Fluoride	Probiotic vs Fluoride	*P* Value*
27.36 ± 5.67 29.58 ± 5.95	−2.23 (−2.44 to −2.00)	<.001	−0.53 (−3.57 to 2.51)	−1.99 (−4.8 to 0.82)	−1.46 (−4.74 to 1.82)	.467

Table 4. Intergroup Comparison of Patient-Reported Outcomes

Outcome	Scale	Aloe Vera Mouthwash (n = 30), n (%)	Probiotic Mouthwash (n = 30), n (%)	Fluoride Mouthwash (n = 30), n (%)	*P* Value*
Burning sensation	No	17 (56.7)	23 (76.7)	23 (76.7)	.512
	light	7 (23.3)	5 (16.7)	4 (13.3)	
	moderate	3 (10.0)	2 (6.7)	2 (6.7)	
	severe	3 (10.0)	0 (0)	1 (3.3)	
Taste	No	4 (13.3)	7 (23.3)	11 (36.7)	.393
	light	14 (46.7)	13 (43.3)	14 (46.7)	
	moderate	7 (23.3)	6 (20.0)	3 (10.0)	
	severe	5 (16.7)	4 (13.3)	2 (6.7)	
Tooth staining	No	29 (96.7)	24 (80.0)	27 (90.0)	.249
	light	1 (3.3)	5 (16.7)	2 (6.7)	
	moderate	0 (0)	1 (3.3)	1 (3.3)	
	severe	0 (0)	0 (0)	0 (0)	

* Fisher exact test.

with other orthodontic clinical trials could be made due to lack of previous research.

Patient adherence to following instructions regarding mouthwash rinsing was above 95% across study groups of the trial (Figure 2). Similar studies[4,5,25,26] have recorded lower levels, with Herrera et al.[26] reporting 16% failed mouthwash intake and Rioboo et al.[25] reporting 75% usage after 1 month. The most notable methodological difference in the current study, which could account for this, was the successful utilization of mobile applications. Hence, present-day mobile applications to ensure patient compliance in clinical practice should be a new norm.

Irrespective of the efficacy of a mouthwash, eventual success will also depend on patient acceptance of the product and any adverse effects. In this respect, the response of patient-reported outcomes regarding burning sensation, taste, and tooth staining tendencies was critical. Since no differences were found among groups in the frequency of responses to these outcomes, it could be inferred that the overall acceptability of all three mouthwashes in this regard was similar. Also, none of the responses were in the very severe category in any mouthwash group (Table 4).

Probiotic genera *Lactobacillus* and *Bifidobacterium* may be involved in deep caries and the progression of caries.[21] Hence *Saccharomyces boulardii*, the only probiotic species of yeast, was used as probiotic intervention in the current study. It is also safe for use in a healthy population.[27]

The trial had a few shortcomings, including the short duration with patients recruited from a single-center, teaching hospital. Also, the study employed standard-of-care treatment as a control group due to the ethical concerns raised by the approving authority.[28]

CONCLUSIONS

- No significant difference was found in efficacy among the three mouthwashes: aloe vera, probiotic, and fluoride, in reducing S. mutans level in plaque.
- Qualitative and sensory assessments concerning burning sensation, taste, and tooth staining found no significant differences among the three mouthwashes.
- Smartphone-based applications can help improve patient compliance.

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