Comparative Evaluation of the Antimicrobial Effects of Different Mouthrinses against Oral Pathogens: An In Vitro Study

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ABSTRACT
Aim: To assess the antimicrobial effects of natural and semi-natural mouthrinses on isolates of Streptococcus mutans, Lactobacillus fermentum, and Lactobacillus casei obtained from the saliva samples and their reference strains.

Materials and methods: Natural and semi-natural mouthrinses included in this study were herbal mix mouthrinse, cranberry mouthrinse, chlorhexidine digluconate mouthrinse, cranberry extract mixed with chlorhexidine digluconate mouthrinse, chlorhexidine digluconate mouthrinse with alcohol (positive control), and distilled water (negative control). The microbiological examination tests were minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition test for the saliva isolates of S. mutans, L. fermentum, and L. casei.

Result: Compared with distilled water, herbal mix, cranberry, cranberry mixed with chlorhexidine, chlorhexidine with alcohol (+), and chlorhexidine mouthrinses were associated with a significant increase of the zone of inhibition 34.354, 34.255, 34.219, 10.801, and 9.386, respectively. Both MIC and MBC were significantly higher in the cranberry mixed with chlorhexidine than in chlorhexidine with alcohol. The MIC and MBC of mouthrinses were significantly lower in the S. mutans and L. fermentum than in L. casei.

Conclusion: Herbal mix and cranberry mouthrinses could be effective natural alternative to chlorhexidine mouthrinse with or without alcohol in improving oral health.

Clinical significance: Different mouthrinses proposed in this study showed antimicrobial effects against the tested oral pathogens, and possibly the tested mouthrinses will lead for future formulation of natural or semi-natural pharmaceutical mouthrinses.

Keywords: Chlorhexidine, Lactobacillus, Microbiology, Mouthrinses, Streptococcus mutans.

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INTRODUCTION
Streptococcus mutans (S. mutans) is a gram-positive bacterium. It is a significant contributor to the development of cariogenic plaque.1,2 Lactobacillus is a genus of gram-positive bacterium associated with caries progression and represent one of the main parts of the lactic acid bacteria group.3 However, a few number of children with no caries were positive for lactobacilli.4–6 Lactobacillus genus includes more than 80 species, some of which have been found in the oral cavity such as Lactobacillus fermentum and Lactobacillus casei groups.6–12 In clinical practice, the use of preventive measures is critical to decrease the risk for dental caries in highly susceptible individuals. It is believed that early prevention of bacterial growth can eliminate its colonization which aid in prevention of destruction of the tooth structure.13 Practicing oral hygiene by teeth brushing as a mechanical plaque control measure is desirable and commonly used.14 Besides that, many anti-plaque agents have been in use as supplementary aids.14 It is believed that using mouthrinses could act as an effective and harmless method for delivery of antimicrobial agents that prevent bacterial adhesion, colonization, and disturb the bacterial growth.14 Chlorhexidine is the gold standard mouthrinse due to its antimicrobial action.15,16 However, despite its side effects such as teeth and soft tissue discoloration, taste alteration, and supragingival calculus formation, its continued use is supported.15–18 It was reported that the existence of plaque covering the teeth surfaces increased the chlorhexidine side effects and authors reinforced the importance of plaque removal before chlorhexidine mouthrinse usage.19 Nowadays, patients are more worried about the side effects of synthetic chemical products on their oral health. Moreover, growing connection between oral health and herbal remedy offers an enduring approach of health repair in a most valuable way.20 To the best of our knowledge, there

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are no investigations concerning the antimicrobial effects of the combination of naturally derivative compounds such as herbal mix mouthrinse and semi-natural compounds such as cranberry extract mixed with chlorhexidine digluconate mouthrinse against oral pathogens. Therefore, the aim of this in vitro comparative study was to assess the antimicrobial effects of different natural and semi-natural mouthrinses on isolates of S. mutans, L. fermentum, and L. casei that were obtained from the saliva of the 7- to 10-year-old Saudi children and their reference strains ATCC 25175, ATCC 9338, and ATCC 334, respectively. The null hypothesis was there is no significant difference in the antimicrobial effects between tested and control mouthrinses against S. mutans, L. fermentum, and L. casei that were isolated from the saliva of the 7- to 10-year-old Saudi children.

**Materials and Methods**

Twenty children who met the inclusion criteria were selected from the Dental University Hospital, King Saud University, after study approval by the Institutional Review Board. A consent was obtained from parents or legal guardian.

**Inclusion Criteria**

- Healthy Saudi 7- to 10-year-old children with American Society of Anesthesiology (ASA) I who have positive or definitely positive behavior\(^2\) and have at least four decayed, missing, and/ or filled teeth due to caries (DMFT/dmft ≥ 4) with high decayed component.\(^2\)
- Children adhering to at least once daily toothbrushing routine without practising other oral hygiene measures and have minimal dental plaque and gingivitis as evaluated using a modification of hygiene index and the simplified gingival index.\(^2\)

**Exclusion Criteria**

Children with medical history that could affect the conduct of the study, with history of antibiotic or mouthrinses usage (past 1 month), abscess, cellulitis, or other conditions requiring emergency treatment, history of less than 1 week after professional fluoride application, and children with (DMFT/dmft ≥ 4) but had all carious teeth restored.\(^2\)\(^4\)\(^-\)\(^6\)

**Study Design**

Unstimulated saliva (2 mL) was collected in aseptic condition into plastic disposable tube. Saliva samples were cultured with a swab using the spread plate method and specific media Mutans-Sanguis (MS) agar for S. mutans, Lactobacilli MRS agar for L. fermentum and L. casei in anaerobic condition using AnaeroPack-Anaero at 37°C for 48 hours. A total of 40 plates were cultured, 20 plates with MS agar for S. mutans and 20 plates with Lactobacilli MRS agar for L. fermentum and L. casei. The media were prepared in accordance with the manufacturer’s instructions (HiMedia Laboratories Pvt. Ltd, Mumbai, India). After the incubation period, the suspected colonies of S. mutans, L. fermentum, and L. casei were further identified for confirmation using an automated system (Vitek*) of biochemical identification (BioMerieux SA, Marcy-l’Etoile, France). This laboratory study included 288 samples divided into 72 samples of reference strains of the three pathogens S. mutans ATCC 25175, L. fermentum ATCC 9338, and L. casei ATCC 334 that were obtained from KWIK-STIK™; 24 samples for each pathogen that were randomly divided into six major groups according to the different tested mouthrinses and control groups planned to use to evaluate the zone of inhibition; and 4 samples per rinse. The other 216 samples were conducted on the saliva isolates of the three oral pathogens S. mutans, L. fermentum, and L. casei, 72 samples for each oral pathogen that were randomly divided into six major groups according to the different tested mouthrinses and control groups planned to use to evaluate the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and zone of inhibition; 24 samples per microbiological test; and 4 samples per rinse. The different mouthrinses included in this study were herbal mix mouthrinse (1%), cranberry mouthrinse (0.6%), chlorhexidine digluconate mouthrinse (0.12%), cranberry extract (0.3%) mixed with chlorhexidine digluconate mouthrinse (0.06%), chlorhexidine digluconate mouthrinse (0.12%) with alcohol (positive control), and distilled water (negative control). The microbiological examination tests evaluated were MIC, MBC, and zone of inhibition test on 216 samples for the saliva isolates of three oral pathogens S. mutans, L. fermentum, and L. casei while zone of inhibition test only on 72 samples of reference strains of three pathogens S. mutans ATCC 25175, L. fermentum ATCC 9338, and L. casei ATCC 334.

**Sample Size Calculation**

nQuery Sample Size Software (nQuery Advanced 8.2; Statsols, Cork, Ireland) was used to calculate sample size for reference strains and saliva isolates. When the zone of inhibition test was measured in two types of strains (reference strains and saliva isolates), six mouthrinses of size 4 each, and three pathogens (n = 144), a one-way analysis of variance would have 80% power to detect at the 5% level a difference in means of zone of inhibition test characterized by a variance of means of 13.98, assuming that the common standard deviation is 14.3. The two other microbiological tests, MIC and MBC, were measured in one type of strain (saliva isolate samples), six mouthrinses, and three oral pathogens of size 4 each (one original test that had repeated triplicate times for confirmation).

**Blindness**

Different individuals were assigned to help in creating unbiased research environment.

**Compositions of Mouthrinses and Methods of Extraction**

- Herbal mix mouthrinse (1%) is made from 0.36% pomegranate peel extract (PPE), 0.36% myrrh resin extract, 0.18% chamomile flower extract, 0.1% cinnamon bark extract, 0.1 g sodium benzoate, and 0.2 g ascorbic acid.
- Preparation of Pomegranate Peels Powder
  Pomegranates having no visible external cuts were obtained from local markets, fruits were washed with water, cut, and the arils and seeds were removed. Peels were cut into pieces, dried at 50°C in a convection oven for 3 days. The dried peels were ground with pestle and mortar to powder of 1 mm size.
- Preparation of Myrrh, Chamomile Flower, and Cinnamon Bark Powder
  Herbs were obtained from local markets, washed, and ground to yield powder of 1 mm size.
- Cranberry mouthrinse (0.6%) is made from cranberry extract dissolved in sterilized water with other ingredients (0.1 g ZnCl\(_2\), 0.1 g sodium saccharine, 0.05 g menthol, 0.1 g sodium benzoate, and 3 mL of glycerin).
Preparation of Cranberry Powder
Fresh cranberry was obtained from market, rinsed, cut in half to remove seeds, dried in the oven at 40°C. Then ground to powder of 1 mm size.

Preparation of the Extracts
A 10 g of prepared powders was extracted with 300 mL of sterilized water by soaking and stirring for 24 hours, then filtered through Whatman filter paper No. 41 (GE Healthcare, Little Chalfont, UK), and evaporated to dryness at 40°C in an oven for 12 hours in the dark, and the obtained residue was stored in a dark container at 4°C. The used powder was 10 g for the pomegranate peel, myrrh, chamomile, cinnamon bark, and cranberry. While the yield extract was 2.4, 4, 1.4, 0.4, and 2 g, respectively.

• Chlorhexidine digluconate mouthrinse (0.12%) (Kin®, Barcelona, Spain) was obtained from the market.
• Cranberry extract 0.3% mixed with chlorhexidine digluconate mouthrinse 0.06%.
• Chlorhexidine digluconate mouthrinse (0.12%) containing alcohol (11% ethanol)–positive control.
• Distilled water mouthrinse–negative control.

Microbiological Examination Tests

MIC
The MIC of tested mouthrinses against oral pathogens was assessed by agar dilution method as recommended by the Clinical and Laboratory Standards Institute.27,28

MBC
The MBC of tested mouthrinses was determined from MIC range using spread plate method.28

Zone of Inhibition
The antibacterial activity was estimated using disk diffusion method (Kirby–Bauer) on agar plate. Zone of inhibitions were measured for each mouthrinse using caliper by two external evaluators.4,29

Reliability and Statistical Analysis
The intra-rater agreement approach was used to assess the internal consistency of a single rater for measuring the DMFT index of 20 children. This approach showed an excellent intra-rater agreement with intra-rater coefficient (ICC) of 0.91 and 95% confidence interval (CI): 0.696–0.978. The inter-rater agreement approach was assessed on 144 samples (both types of strains: reference strains and saliva isolate samples) for measuring the zones of inhibition, which were evaluated by two blinded raters. The inter-rater agreement between the two raters was perfect, ICC of 0.975 and 95% CI: 0.966–0.982 in both types of strain groups. The related samples of the Wilcoxon signed-rank test demonstrated no significant differences in measuring the zone of inhibition between the two raters (p = 0.997). The SAS software version 9.4 (SAS Institute, Inc., Cary, NC, USA) was used for data analysis. The zone of inhibition test was analyzed using a robust analysis of variance based on M estimation. Robust analysis of variance based on M estimation and Kruskal–Wallis tests were used to analyze the MIC and MBC. An α = 5% was used for significance of all analyzes.

Results
The study included 20 children, 9 (45%) females and 11 (55%) males with age (mean ± standard deviation) 8.15 ± 1.09 years and range 7–10 years as well as DMFT 7.65 ± 1.84 and range 5–11. We used robust analysis of variance based on M estimation and Kruskal–Wallis to analyze the MIC (Table 1) and the MBC (Table 2). In regard to zone of inhibition test, a total of 144 samples (number/percent): 72/50% saliva isolate samples and 72/50% reference strain samples were analyzed regarding the six experimental and control mouthrinses each 24/16.67% and the three oral pathogens each 48/33.33%. The effects of type of strains, mouthrinses, and oral pathogens on zone of inhibition were assessed (Table 3). We found that type of strains, mouthrinses, oral pathogens, and their interactions explain 94.5% of the variance in the zone of inhibition. This indicates that the model explains the variability in the zone of inhibition well. Compared with distilled water (Table 3), herbal mix, cranberry, cranberry mixed with chlorhexidine, chlorhexidine with alcohol (+), and chlorhexidine mouthrinses were associated with a significant increase in the zone of inhibition 34.354 (p = 0.001), 34.255 (p = 0.001), 34.219 (p = 0.001), 10.801 (p = 0.001), and 9.386 (p = 0.001), respectively (Figs 1A and 2). The S. mutans was associated with an increase of 1.554 (p = 0.022) in the zone of inhibition compared to L. casei (Fig. 1B). There was no significant effect of L. fermentum on the zone of inhibition 1.053 (p = 0.120) compared to L. casei. There was evidence that the effect of mouthrinses on the zone of inhibition varied by type of strains (Table 3). Compared with the reference strain samples, saliva isolate samples were associated with an increase in the zone of inhibition of 5.417 (p = 0.001) in herbal mix mouthrinse and 1.991 (p = 0.006) in cranberry mouthrinse with a decrease in the zone of inhibition of 1.851 (p = 0.011) in chlorhexidine with alcohol (Fig. 3A). There was no evidence that chlorhexidine (p = 0.286) or cranberry mixed with chlorhexidine (p = 0.848) varied by type of strains. We also observed significant interaction effects between mouthrinses and oral pathogens on the zone of inhibition (Table 3). In chlorhexidine, there was a significant increase in the zone of inhibition of 8.346 (p = 0.001) in S. mutans compared to L. casei. Additionally, in chlorhexidine with alcohol (+), there was a significant increase in the zone of inhibition of 4.632 (p = 0.001) in S. mutans compared to L. casei (Fig. 3B). However, in cranberry mixed with chlorhexidine, there was a significant decrease in the zone of inhibition of 10.327 (p = 0.001) in S. mutans and a decrease of 5.679 (p = 0.001) in L. fermentum compared to L. casei (Fig. 3B). For each oral pathogen, the MIC and MBC of mouthrinses were assessed for saliva isolate samples. The Kruskal–Wallis test demonstrates that the mouthrinse has a significant effect on the MIC and MBC in S. mutans, L. fermentum, and L. casei (p < 0.05). The robust analysis of variance demonstrates that the MIC and MBC were significantly higher in the cranberry mixed with chlorhexidine group than in chlorhexidine with alcohol (+) (p = 0.0012) (Fig. 4 and Tables 1 and 2). The R² value was moderate 0.593; it indicates that this model explains the variability in the MIC and MBC to be moderate. The MIC and MBC were significantly lower in the S. mutans and L. fermentum groups than in L. casei (p < 0.05) (Fig. 4). Figure 5 shows the distribution of the MIC and MBC by oral pathogens. Most of L. fermentum were inhibited in their growth in 0.000001 MIC and 0.000001 MBC compared to other oral pathogens. However, most of L. casei were inhibited in their growth in 0.001 MIC and 0.01 MBC compared to other oral pathogens. The inference of the study is
that using different mouthrinses proposed in this research showed antimicrobial effects against the tested oral pathogens, and possibly the tested mouthrinses will lead for future formulation of natural or semi-natural pharmaceutical mouthrinses. Moreover, using different mouthrinses proposed in this research provide objective data for future research to challenge production of more effective caries-preventive agents.

**DISCUSSION**

The null hypothesis was rejected, as there was a significant difference in the antimicrobial effects between tested and control mouthrinses against *S. mutans, L. fermentum*, and *L. casei* that were isolated from the saliva of the 7- to 10-year-old Saudi children. *S. mutans* and lactobacilli are key bacterial species implicated in the etiology of caries. In a study conducted by Wen et al., they suggested that the relations between *S. mutans* and lactobacilli are species specific, and this could affect the cariogenic potential of the community. In our study, compared with distilled water, herbal mix, cranberry, cranberry mixed with chlorhexidine, chlorhexidine with alcohol (+), and chlorhexidine mouthrinses were associated with a significant increase of 34.354, 34.255, 34.219, 10.801, and 9.386, respectively, in the zone of inhibition. This result could be due to the component of the tested rinses. Herbal mix mouthrinse was made mainly from PPE and myrrh resin extract. Pomegranate peel extract mouthrinse has notable antimicrobial activity against *S. mutans* and can be used to prevent dental caries. Moreover, an in vitro study was conducted by Mehta et al. to evaluate the efficacy of pomegranate peel, lotus leaf, guava leaf, and coffee extracts on oral microorganisms reported that pomegranate peel and lotus have the maximum efficacy against *S. mutans* and *S. mitis*. Another study conducted by Kote et al. found that there was a significant reduction in the number of colony-forming units of streptococci and lactobacilli after pomegranate rinse.

A recent clinical study conducted by Singla and his colleagues to evaluate and compare the antibacterial efficacy of different mouthrinses prepared from pomegranate, grape seed, and guava extracts against oral streptococci reported that there is a significant reduction in the colony-forming unit of oral streptococci after the usage of pomegranate and guava mouthrinses for 7 days. In respect to myrrh, Eid et al. reported the capability of myrrh plant extract to inhibit the growth of *S. mutans* with a clear zone of 11 mm around the disk. Another recent study was conducted by Park and Yoon to explore the antimicrobial activity of plant-derived essential oils against *S. mutans*, *Porphyromonas gingivalis*, and *L. rhamnosus* showed that myrrh oil had antimicrobial effect against the aforementioned three bacterial strains with the highest antimicrobial activity at 18.34 mm zone of inhibition against *S. mutans*. Yemeni myrrh was also evaluated by Almekhlafi et al., and the result showed a notable antimicrobial activity of myrrh mouthrinse against *Staphylococcus aureus*, *S. mutans*, and *Candida albicans*. Some investigators continue trying to find safe

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**Table 1:** The effects of mouthrinses and oral pathogens on minimum inhibitory concentration dilution in saliva isolate samples

| Oral pathogens | Mouthrinse | B     | SE    | Chi-square | p     |
|----------------|------------|-------|-------|------------|-------|
| *S. mutans*    | Herbal     | 0.0008| 0.0001| 72.94      | <0.0001|
| *S. mutans*    | Cranberry  | 0.0001| 0.0001| 0.64       | 0.4232 |
| *S. mutans*    | Chlorhexidine | 0   | 0.0001| 0.07       | 0.7895 |
| *S. mutans*    | Cranberry mixed with chlorhexidine | 0.0004| 0.0001| 10.43      | 0.0012 |
| *S. mutans*    | Chlorhexidine with alcohol (+) | 0   |       |           |       |
| *L. casei*     | Herbal     |       |       |           |       |
| *L. casei*     | Cranberry  |       |       |           |       |
| *L. casei*     | Chlorhexidine |       |       |           |       |
| *L. casei*     | Cranberry mixed with chlorhexidine |       |       |           |       |
| *L. casei*     | Chlorhexidine with alcohol (+) |       |       |           |       |

SE, standard error
compounds that could prevent and obstruct biofilm formation. In this regard, it was reported by Bonifait and Grenier that the high-molecular-weight polyphenols extracted from cranberries could limit dental caries by preventing biofilms formation and inhibit the production of organic acids by cariogenic bacteria. In our study, *S. mutans* was associated with an increase of 1.554 in the zone of inhibition compared to *L. casei*. Lactobacilli display a wide range of antibiotic resistance naturally. D’Aimmo reported that lactobacilli are resistant to nalidixic acid, aztreonam, cycloserin, kanamycin, metronidazole, polymyxin B, and spectinomycin and susceptible to rifampicin, bacitracin, clindamycin, erythromycin, novobiocin, and penicillin. In specific, *L. casei* demonstrated high resistance to aztreonam, cycloserine, polymyxin B and vancomycin. 

### Table 3: The effects of type of strains, mouthrinses, and oral pathogens on zone of inhibition

|                          | B      | SE     | Chi-square | p       |
|--------------------------|--------|--------|------------|---------|
| Intercept                | 5.298  | 0.532  | 99.0       | 0.001*  |
| Type of strain           |        |        |            |         |
| Saliva                   | 1.405  | 0.591  | 5.7        | 0.017*  |
| Herbal Mouthrinse        | 34.354 | 0.723  | 2256.0     | 0.001*  |
| Cranberry Mouthrinse     | 34.255 | 0.723  | 2242.9     | 0.001*  |
| Chlorhexidine Mouthrinse | 9.386  | 0.723  | 168.4      | 0.001*  |
| Cranberry mixed with chlorhexidine Mouthrinse | 34.219 | 0.723 | 2238.3 | 0.001* |
| Chlorhexidine with alcohol (+) Mouthrinse | 10.801 | 0.723 | 223.0 | 0.001* |
| Oral pathogens           |        |        |            |         |
| *S. mutans*              | 1.554  | 0.677  | 5.3        | 0.022*  |
| *L. fermentum*           | 1.053  | 0.677  | 2.4        | 0.120   |
| Mouthrinse × type of strain |        |        |            |         |
| Herbal Saliva            | 5.417  | 0.723  | 56.1       | 0.001*  |
| Cranberry Saliva         | 1.991  | 0.723  | 7.6        | 0.006*  |
| Chlorhexidine Saliva     | −0.772 | 0.723  | 1.1        | 0.286   |
| Cranberry mixed with chlorhexidine Saliva | −0.138 | 0.723 | 0.0    | 0.848   |
| Chlorhexidine with alcohol (+) Saliva | −1.851 | 0.723 | 6.6     | 0.011*  |
| Type of strain × oral pathogens |        |        |            |         |
| Saliva *S. mutans*       | −2.733 | 0.511  | 28.6       | 0.001*  |
| Saliva *L. fermentum*    | −1.856 | 0.511  | 13.2       | 0.001*  |
| Oral pathogens × Herbal  |        |        |            |         |
| Saliva *S. mutans*       | −0.125 | 0.886  | 0.0        | 0.888   |
| Oral pathogens × Herbal  |        |        |            |         |
| Saliva *L. fermentum*    | 1.479  | 0.886  | 2.8        | 0.095   |
| Oral pathogens × Herbal  |        |        |            |         |
| Cranberry *S. mutans*    | 0.400  | 0.886  | 0.2        | 0.651   |
| Cranberry *L. fermentum* | −0.510 | 0.886   | 0.3        | 0.565   |
| Oral pathogens × Herbal  |        |        |            |         |
| Cranberry *S. mutans*    | 8.346  | 0.886  | 88.8       | 0.001*  |
| Oral pathogens × Herbal  |        |        |            |         |
| Cranberry *L. fermentum* | −0.313 | 0.886   | 0.1        | 0.724   |
| Oral pathogens × Herbal  |        |        |            |         |
| Cranberry mixed with chlorhexidine *S. mutans* | −10.327 | 0.886 | 135.9    | 0.001*  |
| Oral pathogens × Herbal  |        |        |            |         |
| Cranberry mixed with chlorhexidine *L. fermentum* | −5.679 | 0.886 | 41.1    | 0.001*  |
| Oral pathogens × Herbal  |        |        |            |         |
| Chlorhexidine with alcohol (+) *S. mutans* | 4.632  | 0.886 | 27.3    | 0.001*  |
| Oral pathogens × Herbal  |        |        |            |         |
| Chlorhexidine with alcohol (+) *L. fermentum* | −0.567 | 0.886 | 0.4     | 0.522   |

*Significant; SE, standard error

Figs 1A and B: (A) The effects of mouthrinses on the zone of inhibition. (B) The effects of oral pathogens on the zone of inhibition
saliva isolate samples were associated with an increase in the zone of inhibition of 5.417 in herbal mix mouthrinse, an increase of 1.991 in cranberry mouthrinse, and a decrease in the zone of inhibition of 1.851 in chlorhexidine with alcohol. This could be a good sign that indicates the susceptibility of oral pathogens isolated from Saudi children saliva to active compounds of herbal

Figs 3A and B: (A) The effects of mouthrinses on the zone of inhibition by type of strains. (B) The effects of mouthrinses on the zone of inhibition by oral pathogens

Figs 2A to D: (A) Zone of inhibition of herbal mix mouthrinse on S. mutans. (B) Zone of inhibition of cranberry mouthrinse on L. fermentum. (C) Zone of inhibition of cranberry extract mixed with chlorhexidine digluconate mouthrinse on L. casei. (D) Zone of inhibition of distilled water on L. fermentum
extracts and proanthocyanidins and flavonols constituents of cranberry. The effects of the extracts of flavonols, anthocyanins, and proanthocyanidins from the cranberry on virulence factors involved in *S. mutans* biofilm development and acidogenicity showed inhibition of the surface-adsorbed glucosyltransferases and F-ATPases activities, and the acid production by *S. mutans*.43 Philip et al. reported that a highly purified polyphenol-rich cranberry extract was able to significantly disrupt acidogenicity, metabolic activity, exopolysaccharide/microbial biovolumes, and structural organization of *S. mutans* biofilms without affecting bacterial viability.44 In regard to pomegranate, the antimicrobial agents (tannins) form high-molecular-weight complex that can aid in microorganisms lysis and obstruct bacterial adherence orally.45 In our study, chlorhexidine with and without alcohol mouthrinses exhibited a significant increase in the zone of inhibition against *S. mutans* compared to *L. casei*. This is supported by other studies stated that chlorhexidine is considered a potent agent against *S. mutans*. However, *L. casei* has shown some resistance to chlorhexidine.46 Besides that, this study showed that the MIC and MBC were significantly higher in the cranberry mixed with chlorhexidine group than in chlorhexidine with alcohol (p = 0.0012), and this could be due to the decreased concentrations of cranberry (0.3%) and chlorhexidine (0.06%) that were used in the semi-natural rinse. The findings in our study should be interpreted in the context of some limitations including its *in vitro* setting. Our *in vitro* methodology does not reflect the complex polymicrobial, ecological, and environmental influences encountered in the oral cavity. *In vitro* studies lack reproduction of oral environment. Nevertheless, *in vitro* studies can provide valuable data of some variables with no interference from other factors. However, in
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spite of these limitations, the research does designate a number of positive links between in vitro effect and clinical effect. Well-designed clinical trials are warranted to demonstrate whether the beneficial properties shown in our study can translate into efficient and valuable therapeutic approaches for caries prevention.

**CONCLUSION**

Within the limitations of this in vitro study, it can be concluded that herbal mix and cranberry mouthrinses could be effective natural alternative to chlorhexidine mouthrinse with or without alcohol in improving oral health.

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**REFERENCES**

1. Chandri R, Banthia P, Banthia R. Biofilms: a microbial home. J Indian Soc Periodontol 2011;15(2):111–114.
2. Hamada S, Slade H. Biology, immunology, and cariogenicity of Streptococcus mutans. Microbiol Rev 1980;44(2):331–384.
3. Cautfield P, Schönh C, Sariaithong P, et al. Oral lactobacilli and dental caries: a model for niche adaptation in humans. J Dent Res 2015;94(9 Suppl):110–118.
4. Leverett D, Proskin H, Featherstone J, et al. Caries risk assessment in a longitudinal discrimination study. Journal of dental research 1993;72(2):538–543.
5. Marchant S, Brailsford SR, Twomey AC, et al. The predominant microflora of nursing caries lesions. Caries Res 2001;35(6):397–406.
6. Piwat S, Teanapaip P, Thitasomakul S, et al. Lactobacillus species and genotypes associated with dental caries in Thai preschool children. Mol Oral Microbiol 2010;25(2):157–164.
7. Coeuret V, Dubernet S, Bernardeau M, et al. Isolation, characterisation and identification of lactobacilli focusing mainly on cheeses and other dairy products. Le Lait, INRA Editions 2003;83(4):269–306.
8. Mitrakul K, Vongsavan K, Suratanaichak P. Prevalence of Streptococcus mutans and Lactobacillus fermentum and their association with caries and dietary habits in preschool Thai children. Eur Arch Paediatr Dent 2013;14(2):83–87.
9. Shimada A, Noda M, Matoba Y, et al. Oral lactic acid bacteria related to the occurrence and/or progression of dental caries in Japanese preschool children. Biosci Microbiota, Food and Health 2015;34(2):29–36.
10. Li Y, Argimón S, Schönh CN, et al. Characterizing diversity of Lactobacillus associated with severe early childhood caries: a study protocol. Adv Microb 2015;5:9–20.
11. Nancy J, Dorigrac G. Lactobacilli from the dentin and saliva in children. J Clin Pediat Dent 1992;16(2):107–111.
12. Botha SJ, Boy SC, Botha FS, et al. Lactobacillus species associated with active caries lesions. J Dent Assoc S Afr 1998;53(1):3–6.
13. Jothika M, Vanajassum P, Someshwar B. Effectiveness of probiotic, chlorhexidine and fluoride mouthwash against Streptococcus mutans – Randomized, single – blind, in vivo study. J Int Soc Prev Community Dent 2015;5(1):1–7.
14. Sharma U, Jain RL, Pathak A. A clinical assessments of effectiveness of mouthwashes in comparison to toothbrushing in children. J Indian Soc Pedod Prev Dent 2004;22(2):38–44.
15. Van der Weijden FA, Van der Slujs E, Cinacio SG, et al. Can chemical mouthwash agents achieve plaque/gingivitis control? Dent Clin North Am 2015;59(4):799–829.
16. James P, Worthington HV, Parnell C, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev 2017;3(3):CD008676.
17. Flitòta L, Gjerme P, Rölla G, et al. Side effects of chlorhexidine mouthwashes. Scand J Dent Res 1971;79(2):119–125.
18. Ernst C, Canbek K, Dillenburger A, et al. Clinical study on the effectiveness and side effects of hexitidine and chlorhexidine mouthrinses vs a negative control. Quintessence Int 2005;36(8):641–652.
19. Zanatta F, Antoniazzi R, Rössing C. Staining and calculus formation after 0.12% chlorhexidine rinses in plaque-free and plaque covered surfaces: a randomized trial. J Appl Oral Sci 2010;18(5):515–521.
20. Dabholkar CS, Shah M, Kathariya R, et al. Comparative evaluation of antimicrobial activity of pomegranate-containing mouthwash against oral-biofilm forming organisms: an invitro microbial Study. J Clin Diagn Res 2016;10(3):ZC65–ZC69.
21. Frankel S, Shiere F, Fogels H. Should the parent remain with the child in the dental operatory? J Dent Child 1962;29:150–163.
22. al Ghanim NA, Adenubi JO, Wyne AA, et al. Caries prediction model in pre-school children in Riyadh, Saudi Arabia. Int J Paediatr Dent 1998;8(2):115–122.
23. Lindhe J, Nyman S, Westfelt E, et al. Critical probing depths in periodontal therapy. Compend Contin Educ Dent 1982;3(6):421–430.
24. Loesche WJ, Syed SA. The predominant cultivable flora of carious plaque and carious dentine. Caries Res 1973;7(3):201–216.
25. Klock B, Krasse B. Microbiological and salivary conditions in 9–12-year-old children. Scand J Dent Res 1977;85(1):56–63.
26. Guo L, Shi W. Salivary biomarkers for caries risk assessment. Journal of the California Dental Association 2013;41(2):107–109.
27. https://cslsi.org/media/1928/m07ed11_sample.pdf.
28. Anita P, Sivasamy S, Madan Kumar P, et al. In vitro antibacterial activity of Camellia sinensis extract against cariogenic microorganisms. J Basic Clin Pharm 2015;6(1):35–39.
29. Barry A, Coyle M, Thornsberry C, et al. Methods of measuring zones of inhibition with the BauerKirby disk susceptibility test. J Clin Microbiol 1979;10(5):885–889.
30. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;60(4):353–380.
31. Wen ZT, Liao S, Bitoun JP, et al. Streptococcus mutans Displays altered stress responses while enhancing biofilm formation by lactobacillus casei in mixed-species consortium. Front Cell Infect Microbiol 2017;7:524.
32. Viuda-Martos M, Fernández-Lóaez J, Pérez-alvarez J. Pomegranate and its many functional components as related to human health: a review. Compr Rev Food Sci Food Saf 2010;9(6):635–654.
33. Umar D, Dilshad B, Farhan M, et al. The effect of pomegranate mouthrinse on Streptococcus mutans count and salivary ph: an in vivo study. J Adv Pharm Technol Res 2016;7(1):13–16.
34. Mehta VV, Rajesh G, Rao A, et al. Antimicrobial efficacy of punica granatum mesocarp, nelumbo nucifera leaf, psidium guajava leaf and coffea canephora extract on common oral pathogens: an in vitro study. J Clin Diagn Res 2014;8(7):ZC65–ZC68.
35. Kote SQ, Kote SU, Nagesh L. Effect of pomegranate juice on dental plaque microorganisms (streptococci and lactobacilli). Anc Sci Life 2011;31(2):49–51.
36. Singla S, Malhotra R, N S, et al. Antibacterial efficacy of mouthwash prepared from pomegranate, grape seed and guava extracts against oral streptococci: an in vivo study. J Clin Pediatr Dent 2018;42(2):109–113.
37. Eid H, Assiri Y, Musleh M, et al. An exploration of the effects of Commiphora gilleadenis on Streptococcus mutans biofilm. Saudi J Oral Sci 2015;2(2):74–78.
38. Park C, Yoon H. Antimicrobial activity of essential oil against oral-pathogenic organisms and its many functional components as related to human health: a review. Compr Rev Food Sci Food Saf 2010;9(6):635–654.
39. Almekhlafi S, Thabit A, Alwossabi A, et al. Antimicrobial activity of thyme, carvacrol and oregano mouthwash against oral pathogen. Saudi Pharm J 2015;23(4):271–275.
40. Bonifait L, Grenier D. Cranberry polyphenols: potential benefits for health and oral mucosal surfaces: a randomized trial. Journal of dental research 2013;92:837–853.
42. D’Aimmo MR, Modesto M, Biavati B. Antibiotic resistance of lactic acid bacteria and Bifidobacterium spp. isolated from dairy and pharmaceutical products. Int J Food Microbiol 2007;115(1): 35–42.

43. Duarte S, Gregoire S, Singh AP, et al. Inhibitory effects of cranberry polyphenols on formation and acidogenicity of Streptococcus mutans biofilms. FEMS Microbiol Lett 2006;257:50–56.

44. Philip N, Leishman SJ, Bandara H, et al. Polyphenol-rich cranberry extracts modulate virulence of Streptococcus mutans-Candida albicans biofilms implicated in the pathogenesis of early childhood caries. Pediatr Dent 2019;41(1):56–62.

45. Machado TB, Pinto AV, Pinto MC, et al. In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant Staphylococcus aureus. Int J Antimicrob Agents 2003;21(3):279–284.

46. Emilson C. Potential efficacy of chlorhexidine against Mutans Streptococci and human dental caries. J Dent Res 1999;73(4):682–691.