ORIGINAL RESEARCH

Comparative Evaluation of the Antimicrobial Efficacy of *Elettaria cardamomum* (0.5%) Mouthwash, *Camellia sinensis* (0.5%) Mouthwash, and 0.12% Chlorhexidine Gluconate Mouthwash against *Streptococcus mutans*: An *In Vitro* Study

Sayali Deolikar[1], Ashwin Jawdekar[2], Tanvi Saraf[3], Lakshmi Thribhuvanan[4], Sunnypriyatham Tirupathi[5]

Abstract

Aim: The *in vitro* study aimed to evaluate and compare the antimicrobial efficacy of *Elettaria cardamomum* (0.5%) mouthwash, *Camellia sinensis* (0.5%) mouthwash, and 0.12% chlorhexidine gluconate mouthwash against *Streptococcus mutans*.

Materials and methods: A total of 60 samples of the five mouthwash preparations were prepared to check for their antimicrobial efficacy. The zone of inhibition (ZOI) against *S. mutans* was measured as a diameter in mm, and the minimum inhibitory concentration (MIC) of mouthwash preparations was measured as µg/mL. All the groups were compared statistically using the Mann–Whitney *U* test and the Kruskal–Wallis test.

Results: The highest ZOI was observed in group V chlorhexidine gluconate [mean: 20.8, standard deviation (SD): 0.58], followed by group III *C. sinensis* (alcohol-free) (mean: 15.5, SD: 0.67), group IV *C. sinensis* (alcohol-based) (mean: 14.08, SD: 0.66), and group II *E. cardamomum* (alcohol-based) (mean: 13.2, SD: 0.45). The least ZOI was observed in group I *E. cardamomum* (alcohol-free) (mean: 10.7, SD: 0.45). This difference was statistically significant ($p < 0.01$). The MIC was similar in all the groups ($p = 0.13$).

Conclusion: Chlorhexidine gluconate 0.12% mouthwash showed the best antimicrobial action; however, *C. sinensis* mouthwash showed potential against *S. mutans*. *E. cardamomum* mouthwash exhibited limited antimicrobial activity.

Keywords: Antimicrobial efficacy, *Camellia sinesis* mouthwash, Cardamom mouthwash, Chlorhexidine gluconate mouthwash, *Elettaria*, *Streptococcus mutans*, Minimum inhibitory concentration, Zone of inhibition.

International Journal of Clinical Pediatric Dentistry (2024): 10.5005/jp-journals-10005-2832

Introduction

Dental caries result from a disruption of the balance between hydroxyapatite and oral biofilm.[1,3] *Lactobacillus*, *Streptococcus mutans*, and *Streptococcus sobrinus* are the bacteria mainly involved in the formation of dental caries, among which *S. mutans* is the main causative agent. It is a microorganism capable of virulence under specific environmental conditions.[4,5] Chlorhexidine gluconate is one of the most accepted chemical methods for plaque control. It has a broad-spectrum action against many bacteria, especially mutans streptococci. Chlorhexidine can prevent the adherence of bacteria and metabolism, thus affecting bacterial growth. Mouthwashes provide an efficient and secure mode for delivery of these antimicrobial agents.[6,7]

Though chlorhexidine mouthwashes have a great capability of reducing the *S. mutans* level in saliva, their regular use can result in certain harmful consequences like tooth and tongue staining, drug resistance, desquamation of the oral mucosa, alteration of the taste of the mouth, and dry mouth. To overcome the drawbacks of these chemical antimicrobial agents, herbal agents have been introduced.[8–10] Herbal mouthwashes have been recommended as safe and effective alternatives to synthetic mouthwashes. Many herbal extracts, such as cinnamon, *Salvadora persica* (miswak), *Trachyspermum ammi* (ajwain), tulsi, black myrobalans, neem, green tea, and black tea, have been introduced as herbal antimicrobial agents.[10] *Elettaria cardamomum* (cardamom), known for its very pleasant aroma and taste, also has numerous health benefits. Cardamom has been previously used in mouthwashes as a halitosis-controlling agent, and it has also been proven that extracts of cardamom seeds can inhibit the growth of microorganisms, including *S. mutans*.[11,12] *Camellia sinensis* (green tea leaves) have

[1,4]Department of Pedodontics and Preventive Dentistry, Dr DY Patil Dental College and Hospital, Dr DY Patil Vidyapeeth (Deemed to be University), Pune, Maharashtra, India

[2,3]Department of Pediatric and Preventive Dentistry, Bharati Vidyapeeth Dental College and Hospital, Bharati Vidyapeeth (Deemed to be University), Mumbai, Maharashtra, India

[5]Department of Pedodontics and Preventive Dentistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu; Dr DY Patil Dental College and Hospital, Dr DY Patil Vidyapeeth (Deemed to be University), Pune, Maharashtra, India

Corresponding Author: Sunnypriyatham Tirupathi, Department of Pedodontics and Preventive Dentistry, Saveetha Dental College and Hospital, Saveetha Institute of Medical Sciences (SIMATS), Saveetha University, Chennai, Tamil Nadu, India, Phone: +91 9490549454, e-mail: dr.priyatham@gmail.com

How to cite this article: Deolikar S, Jawdekar A, Saraf T, *et al.* Comparative Evaluation of the Antimicrobial Efficacy of *Elettaria cardamomum* (0.5%) Mouthwash, *Camellia sinensis* (0.5%) Mouthwash, and 0.12% Chlorhexidine Gluconate Mouthwash against *Streptococcus mutans*: An *In Vitro* Study. Int J Clin Pediatr Dent 2024;17(4):461–466.

Source of support: Nil

Conflict of interest: None

antibacterial, anticariogenic, and antioxidative effects. Studies have shown that it is useful in the prevention of dental caries and plaque formation. Barroso et al. (2018) assessed the antimicrobial effect of a green tea and black tea combination on *S. mutans* and stated that the proprietary blend has shown effectiveness against the growth of *S. mutans*.[13] However, there is limited evidence regarding the antimicrobial efficacy of green tea. Many studies have evaluated the antimicrobial efficacy of various herbal mouthwashes against *S. mutans*; however, as stated in the literature, very few studies have been done on the comparative evaluation of the antimicrobial efficacy of cardamom, green tea, and chlorhexidine. Therefore, the study aimed to evaluate and compare the efficacy of *E. cardamomum* (0.5%) mouthwash, *C. sinensis* (0.5%) mouthwash, and chlorhexidine gluconate (0.12%) mouthwash against *S. mutans*.

Materials and Methods

A total of 60 samples were prepared to assess the antimicrobial efficacy of *E. cardamomum* (0.5%) mouthwash (cardamom), *C. sinensis* (0.5%) mouthwash (green tea), and chlorhexidine gluconate (0.12%) mouthwash against *S. mutans*. The samples were divided into five groups.

Preparation of Extract

Fresh, selected *E. cardamomum* seeds and Sencha-type green tea leaves from *C. sinensis* were purchased from the local market of Silvassa, Madhya Pradesh. They were used in the study after identification and taxonomical authentication. They were thoroughly washed first under tap water and then with distilled water. Subsequently, they were air-dried for 48 hours, and fine powder was prepared using a grinding machine. The prepared powders were kept in sterile, airtight amber glass bottles. For the alcohol-free extract of both cardamom and green tea, a cold aqueous method was used with distilled water. A 10% concentration of cold aqueous extract was prepared by suspending the powder in distilled water at a ratio of 1:10 in a flask with a round bottom, and it was kept at 4°C for 72 hours. For the alcohol-based extract of both cardamom and green tea, 70% ethanol was used as a solvent. This mixture was filtered using sterile Whatman No. 1 filter paper. Distilled water and ethanol were used as solvents, respectively, for the preparation of 0.5% alcohol-free and alcohol-based mouthwashes from cardamom extract and green tea extract (Fig. 1).

Fig. 1: Extract of cardamom and green tea

Antimicrobial Assay

Antimicrobial activities of the test and sample mouthwashes were tested using the agar well diffusion method. The minimum inhibitory concentration (MIC) for all the groups was determined by the tube-dilution method following the guidelines of the American Society for Clinical and Laboratory Standards Institute (CLSI). Brain heart infusion (BHI) broth (BHI broth, Hi-Media, Mumbai), a liquid medium, was used according to the dilution method. Extract solutions with concentrations of 8, 4, 2, 1, 0.5, and 0.25 were prepared, and the concentration of each extract was 1 mg/mL. About 500 µL of various concentrations of extract solutions were added to each individual test tube. Then, 500 µL of the bacterial solution (1×10^5 CFU) was added to the mix. Colonies were grown on mitis salivarius bacitracin (MBS) agar (MSB agar, Hi-Media, Mumbai). In the test group, three sets of repetitions were set up in parallel, and average readings were taken for the final results. All the test tubes were incubated at 37°C overnight (16–24 hours), and readings were taken and recorded in the results sheet. Through visual examination, the concentration of the samples without bacterial growth in the solution appeared clear, indicating the MIC of that particular extract against *S. mutans*. Results were recorded. Sterile calibrated graduated autopipettes of different volumes were used (1/0.5 mL, etc.) for transferring extracts and bacterial broth cultures (*S. mutans* ATCC 25175) to each test tube, following aseptic precautions in a laminar flow cabinet. Sterile 7.5 × 1.3 cm test tubes were used for the study.

Results Interpretation

The MIC was expressed as the minimum dilution that reduced the growth, judged by the lack of turbidity in the tube. Thus, no growth was considered the MIC of that particular extract against *S. mutans*, and results were noted accordingly. As a certain amount of faint turbidity may be present in the inoculum itself, the inoculated tube was refrigerated overnight and used as the standard for determining complete inhibition. Confirmation of the MIC for *S. mutans* against each extract was done by subculturing each broth onto sheep blood agar. No growth on the agar medium after overnight incubation at 37°C was considered the MIC for each of the solvents, correlating with the broth, and the growth on the sheep blood agar after 16–24 hours. All the tests were performed in anaerobic conditions.

Measurement of Zone of Inhibition

First, *S. mutans* (ATCC25175) strain was cultured on MSB agar (Figs 1 and 2 and Table 1). Broth culture was prepared using BHI broth. Turbidity was adjusted to 0.5 McFarland standards as per CLSI guidelines. Inoculation of the dry surface of sheep blood agar plates was done by streaking a swab involving the whole surface of sterile agar, rotating the plate approximately 60° each time for an even distribution of the inoculum. This procedure was repeated twice, and in the final step, swabbing of the rim of the agar was performed. The lid was kept open for 3–5 minutes (but not >15 minutes) to allow excess moisture on the surface to be absorbed before transferring the solvent (cardamom extract and green tea extract). Surface plating (lawn culture) was done with respective broth cultures. Wells were made in inoculated agar plates and loaded with the mouthwash at room temperature. These agar plates were then incubated at 37°C for 24 hours. For agar diffusion, 6 mm diameter agar wells were made using a sterile borer. Around 50 µL of 0.5% green tea extract and 0.5% cardamom extracts were placed in the wells in separate Petri dishes (alcohol-free and alcohol-

based extracts). Around 0.12% chlorhexidine was placed in a similar manner. After incubation for 24 hours, the zone of inhibition (ZOI) was measured, and the results were noted in the results table. The zone of inhibition was measured in mm using a measuring scale.

Results

Zone of inhibition was measured as its diameter in mm, and MIC was measured as µg/mL (Figs 3 and 4 and Table 2). The diameter of ZOI of all the 12 samples in group I (*E. cardamomum*, alcohol-free) ranged from 9.4 ± 2 mm to 11.2 ± 2 mm. For group II (*E. cardamomum*, alcohol-based), ZOI ranged from 12.4 ± 2 mm to 14.2 ± 2 mm. For group III (*C. sinensis*, alcohol-free), the diameter of ZOI ranged from 13 ± 2 mm to 16.5 mm. For group IV (*C. sinensis*, alcohol-based), the diameter of ZOI ranged from 12 ± 2 mm to 15.5 ± 2 mm. For group V, it ranged from 19 ± 2 mm to 22.5 ± 2 mm. The MIC of all the 12 samples in group I ranged from 0 to 0.6. For group II, it ranged from 0 to 1.2. For group III, MIC ranged from 0 to 0.6. In group IV, MIC ranged from 0 to 1.2. In group V (0.12% chlorhexidine gluconate), it ranged from 0 to 1.2.

Inferential Analysis

Kruskal–Wallis test [nonparametric analysis of variance (ANOVA) test] followed by a *post hoc* Dunn test was used for the analysis. Additionally, the Mann–Whitney "*U*" test was performed for individual pairwise comparisons. All tests were conducted using a two-sided test at an α level of 0.05. Inferential statistics describe the comparison of the ZOI and MIC of the samples from groups I to V in terms of mean, median, and standard deviation (SD). A comparison

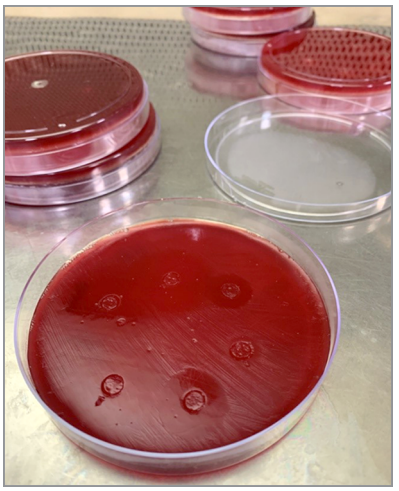

Fig. 3: Zone of inhibition for 0.5% cardamom extract

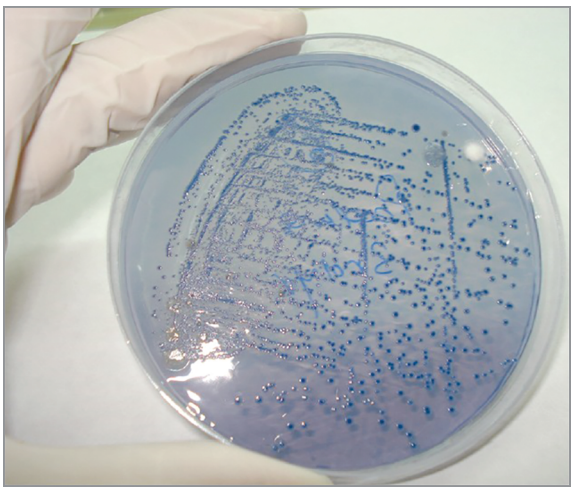

Fig. 2: *S. mutans* cultured on MSB agar

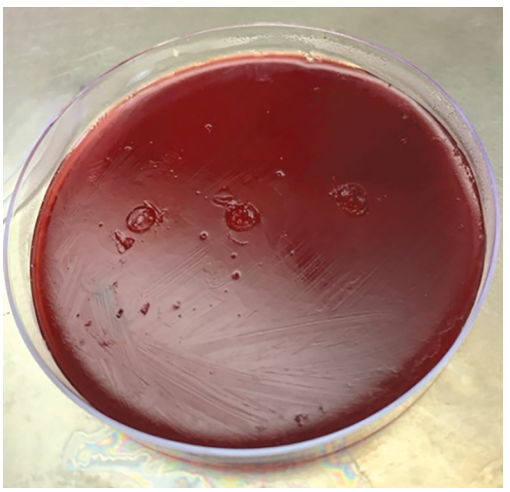

Fig. 4: Zone of inhibition for 0.5% green tea extract

Table 1: Table showing ZOI among all the groups

Group	*n*	*Mean*	*SD*	*Median*	*Minimum*	*Maximum*	*Significance level*
1	12	10.7500	0.4523	11.000	10.0000	11.000	$p < 0.000001$ (significance)
2	12	13.2500	0.4523	13.000	13.0000	14.000	
3	12	15.5833	0.6686	16.000	14.0000	16.000	
4	12	14.0833	0.6686	14.000	13.0000	15.000	
5	12	20.8333	0.5774	21.000	20.0000	22.000	

Table 2: Table showing MIC among all the groups

Group	*n*	*Mean*	*SD*	*Median*	*Minimum*	*Maximum*	*Significance level*
1	12	0.4792	0.07217	0.500	0.2500	0.500	$p = 0.137697$ (nonsignificance)
2	12	0.5833	0.1946	0.500	0.5000	1.000	
3	12	0.5000	0.0000	0.500	0.5000	0.500	
4	12	0.5833	0.1946	0.500	0.5000	1.000	
5	12	0.4792	0.1982	0.500	0.2500	1.000	

of the Mean of ZOI (in mm) was made using the Kruskal–Wallis test and Mann–Whitney "*U*" test. The highest ZOI was for group V (chlorhexidine gluconate) mouthwash, with a mean of 20.8333, a median of 21.000, and an SD of 0.5774. This was followed by group III (*C. sinensis* alcohol-free) mouthwash with a mean of 15.5833, a median of 16.000, and a SD of 0.6686. Group IV (*C. sinensis* alcohol-based) mouthwash showed a mean of 14.0833, a median of 14.000, and an SD of 0.6686, while group II (*E. cardamomum* alcohol-based) mouthwash had a mean of 13.2500, median of 13.000, and SD of 0.4523. The least ZOI was observed with group I (*E. cardamomum* alcohol-free) mouthwash with a mean of 10.7500, median of 11.000, and SD of 0.4523. A *p*-value < 0.000001 ($p < 0.000001$) confirms that the difference was statistically significant, indicating that all groups were not similar with respect to the ZOI. *Post hoc* analysis (Dunn) determined the differences. A comparison of the mean of MIC was done using the Kruskal–Wallis test and the Mann–Whitney "*U*" test. Group I (*E. cardamomum* alcohol-free) mouthwash showed a mean of 0.4792, a median of 0.500, and an SD of 0.07217. Group II (*E. cardamomum* alcohol-based) mouthwash showed a mean of 0.5833, a median of 0.500, and an SD of 0.1946. Group III (*C. sinensis* alcohol-free) mouthwash showed a mean of 0.5000, median of 0.500, and SD of 0.0000. Group IV (*C. sinensis* alcohol-based) mouthwash showed a mean of 0.5833, a median of 0.500, and an SD of 0.1946. Group V (chlorhexidine gluconate) mouthwash showed a mean of 0.4792, a median of 0.500, and an SD of 0.1982. The differences across groups with respect to the MIC were statistically nonsignificant ($p = 0.137697$).

Discussion

Dental caries is a complex disease that begins with a microbial imbalance within the dental biofilm and is associated with factors such as salivary flow and its composition, the level of fluoride exposure, dietary sugar intake, and preventive care strategies.[1–3] *S. mutans*, being the most vital causative agent among all the etiological factors of dental caries, is a microorganism with the capability to acquire various distinct properties, conferring the expression of infectivity determinants, thereby governing its virulence in specific environmental conditions. Through its adherence to the surface, *S. mutans* have the ability to colonize the oral cavity, thereby forming the bacterial biofilm.[14] Auxiliary properties of *S. mutans* colonization include its ability to thrive in an extremely acidic environment and its explicit interlinkage with other bacteria and microorganisms, thereby establishing the ecosystem.[6,15] In children, preventive care strategies play a pivotal role in managing dental caries. However, dental caries is a disease process that must be managed throughout a person's life. Evidence-based practice suggests a holistic trend in clinical practice, shifting from surgical to operative procedures toward a preventive approach to dental caries.[1] There are various preventive strategies used for the prevention of dental caries, including the topical application of various antimicrobial agents such as chlorhexidine, cetylpyridinium chloride, povidone iodine, and silver diamine fluoride. These agents are often applied in the form of gels, varnishes, and mouth rinses. Our study aimed at estimating and comparing the efficacy of 0.12% chlorhexidine mouthwash with herbal mouthwashes, specifically cardamom (0.5%) and green tea (0.5%) mouthwashes, both alcohol-based and alcohol-free, against *S. mutans*.[16] Chlorhexidine gluconate is utilized in a variety of preparations such as mouthwashes, irrigants, medicaments, varnishes, gel-based agents, and sprays. Chlorhexidine gluconate mouthwash is widely available in concentrations of 0.2, 0.12, and 0.1%, as well as in low concentrations of ≤0.06% rinse.[10,16] The effectiveness of chlorhexidine preparation on the oral biofilm is largely dependent on its dosage (Keijser et al.).[17] The maximum dose of chlorhexidine mouthwash preparations is calculated to be 20 mg two times daily, which equals 10 mL of 0.2% chlorhexidine mouthwash (20 mg) or 15 mL of 0.12% chlorhexidine mouthwash (18 mg) (Keijser et al. 2003).[17] Chlorhexidine 0.12% mouthwash (group V) showed the highest ZOI against *S. mutans* compared to other preparations, and this difference was statistically significant (*p*-value < 0.01). This finding supports the literature; a study by Lee et al. assessed the antibacterial activity of chlorhexidine digluconate against *S. mutans* biofilms. They concluded that chlorhexidine significantly decreases bacterial survival in mature *S. mutans* biofilms and resists the reduction in the pH of the culture medium.[18] Walsh et al. published a review in the Cochrane database on chlorhexidine therapies for preventing the occurrence of dental caries in children, adolescents, and young adults. They stated that chlorhexidine has better efficacy than placebo or absence of treatment in preventing dental caries and depleting *S. mutans* levels in children and adolescents.[9] In this study, we compared 0.12% chlorhexidine mouthwash with two herbal mouthwash preparations—cardamom (0.5%) alcohol-free mouthwash, cardamom (0.5%) alcohol-based mouthwash, and green tea (0.5%) alcohol-free mouthwash, and green tea (0.5%) alcohol-based mouthwash. The mean ZOI in these groups was less than that of chlorhexidine 0.12% mouthwash, but these preparations showed antimicrobial efficacy against *S. mutans* growth. After chlorhexidine, 0.12% of the mouthwash group (group V) and group III green tea alcohol-free mouthwash showed significantly high ZOI. This was followed by group IV green tea alcohol-based mouthwash and group II cardamom alcohol-based mouthwash, which showed moderate antimicrobial efficacy. These findings were statistically significant ($p < 0.01$). The aforementioned findings on the antimicrobial efficacy of these agents are consistent with the literature, which suggests their effect on *S. mutans* growth and subsequent plaque formation. Goyal et al. (2017) carried out an assessment and comparison of the antimicrobial effectiveness of green tea as a mouthwash on the amount of colony-forming units of *S. mutans* in children. The authors stated that green tea is efficient as a mouthwash preparation against the *S. mutans* levels and that it has better efficacy in dental plaque compared to saliva. It can be used as an alternative to available mouthwashes.[19] Neturi et al. evaluated the efficacy of green tea rinse compared to water and chlorhexidine on *S. mutans* count. The authors concluded that rinsing with green tea proved to be equally efficient compared to chlorhexidine, which is considered a gold standard.[20] Aneja and Joshi conducted an *in vitro* trial to assess the antibacterial potential of *Amomum subulatum* and *E. cardamomum* seed extracts in resistance to the growth of *S. mutans*, *S. aureus*, *Lactobacillus*, *C. albicans*, and *Saccharomyces cerevisiae*. The ZOI was calculated to be ≥10 mm against the selective organisms. The authors concluded that ethyl alcohol and acetone extracts of seeds of *A. subulatum* and *E. cardamomum* can be utilized as an efficient source of antimicrobial agents used to manage dental caries.[11]

In our study, the least ZOI was observed with cardamom (0.5%) alcohol-free mouthwash (group V). This difference was statistically significant ($p < 0.01$). This information indicates that chlorhexidine mouthwashes show comparatively higher antimicrobial efficacy against *S. mutans* than green tea and cardamom mouthwashes. However, these herbal mouthwashes have also demonstrated a significant reduction in *S. mutans* growth. Herbal mouthwashes

can be effective in certain situations where chlorhexidine would be contraindicated, as chlorhexidine mouthwashes have shown certain adverse effects, as documented in the literature. Haydari et al. stated that with chlorhexidine mouthwash preparations (0.12, 0.2, and 0.06%), altered taste sensations, ulceration and soreness of oral mucosa, tongue, and gingiva, discoloration, and dryness among the participants were recorded in their study. Although not statistically significant, statistically significant differences were observed with "loss of taste" and "numb feeling."[21] Richards (2017) conducted a Cochrane summary review on chlorhexidine mouthwash plaque levels and gingival health, noting that chlorhexidine mouthwash rinsing for 4 weeks or more results in tooth staining.[22] Van Strydonck et al. (2012)[23] observed a systematic review to assess the effectiveness of chlorhexidine mouthwash in establishing dental plaque, staining, and inflammation of the gingiva in patients with gingivitis. They concluded that chlorhexidine rinsing groups demonstrated significant staining.[23] Another disadvantage of chlorhexidine is associated with its broad-spectrum activity. Although evidence is available that chlorhexidine, when used in the estimated concentrations, is considered the gold standard against the development of dental caries-causing bacteria, its action should be taken into consideration with respect to two main hypotheses—the specific plaque hypothesis and the ecological plaque hypothesis. The specific plaque hypothesis focuses on the vitality of specific bacteria in dental plaque. Meanwhile, the ecological plaque hypothesis illustrates that the distinguished bacterial environment provokes the formation of oral microflora, and any imbalance in the microflora can lead to an increase in pathogenic microflora as opposed to the normal healthy oral microflora.[24,25] Marsh (2010) emphasized the importance of the oral ecosystem and explained that the resident microflora of the host not only inhabit placidly at the site but also contribute to maintaining health by enhancing the immune system. Some parts of the acquired microflora can also play a vital role in weakening the strength of immune responses and vanishing the exogenous, which are often pathogenic microorganisms. Antimicrobial agents with broad-spectrum action, such as chlorhexidine, can disrupt the balance of the oral ecosystem by interfering with the normal healthy microflora.[15] Herbal types of mouthwash can potentially overcome the adverse effects caused by chlorhexidine mouthwash. The MIC is the least concentration at which a drug or agent prevents the growth of a bacterium or bacteria. In this particular study, we measured the MIC of all five groups. All groups showed similar MIC against *S. mutans*. This indicates that the minimum concentration at which chlorhexidine (0.12%) mouthwash prevented/inhibited the visible growth of *S. mutans* was similar to the concentration at which cardamom (0.5%) alcohol-free mouthwash, cardamom (0.5%) alcohol-based mouthwash, green tea (0.5%) alcohol-free mouthwash, and green tea (0.5%) alcohol-based mouthwash prevented the visible growth of *S. mutans*. However, there are certain limitations to this study. The most important limitation is that this trial is an *in vitro* trial, and all characteristics of study parameters should be taken into consideration before its use for human trials. *In vitro*, studies can only create a hypothesis and not test one. Additionally, in this particular study, the antimicrobial efficacy of mouthwashes has been assessed only against *S. mutans* (strain ATCC25175) as it is one of the pioneer causative microorganisms involved in the initiation and progression of dental caries. However, various other microorganisms such as *Streptococcus sobrinus*, *Streptococcus sanguis*, *Streptococcus salivarius*, various *Lactobacilli* such as *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Actinomycetes* species such as *Actinomyces viscosus*, *Actinomyces naeslundii* are also involved in the caries process. Therefore, the role of these microorganisms and the effect of these antimicrobial agents on these species should also be tested.[15] Another limitation of these *in vitro* microbiological trials is the potential for contamination of various culture media and transport media, which can lead to errors in the resultant quantitative assessment. Additionally, these procedures are technique-sensitive and require careful handling of materials and equipment, as well as thorough knowledge and expertise in the microbiological field.[33] Although in the presented study chlorhexidine 0.12% mouthwash showed the highest antimicrobial efficacy in terms of ZOI against *S. mutans*, green tea (0.5%) alcohol-free mouthwash, green tea (0.5%) alcohol-based mouthwash, cardamom (0.5%) alcohol-free mouthwash, and cardamom (0.5%) alcohol-based mouthwash have also shown limited but inferential antimicrobial efficacy in terms of ZOI against *S. mutans*. Additionally, all the groups have shown similar MIC against *S. mutans*. Since no trial has been reported on the comparative efficacy of chlorhexidine (0.12%), cardamom (0.5%), and green tea (0.5%) mouthwashes against *S. mutans*, this study is one of a kind and can be considered a foundation for future research.

Conclusion

Chlorhexidine gluconate (0.12%) showed the highest antimicrobial efficacy against *S. mutans* compared to other mouthwash preparations. This was followed by *C. sinensis* (0.5%) alcohol-free mouthwash, which showed high antimicrobial efficacy against *S. mutans*. *C. sinensis* (0.5%) alcohol-based mouthwash, and *E. cardamomum* (0.5%) alcohol-based mouthwashes showed moderate antimicrobial efficacy against *S. mutans*, while *E. cardamomum* (0.5%) mouthwash showed low antimicrobial efficacy against *S. mutans*.

Recommendations

An *in vivo* study with a randomized controlled trial design comparing topical formulations of cardamom and green tea in different age-groups and different caries risk groups can establish the claims we made with respect to antimicrobial efficacy. Green tea has shown antimicrobial potential and could be a replacement for chlorhexidine in certain situations. However, cardamom has shown weak antimicrobial activity against *S. mutans*, and this agent needs further research before its clinical use.

Funding

Self-funded study.

Acknowledgments

The authors express their gratitude to Biotrack Laboratory, Kharghar, and the Department of Microbiology, Shri Vinoba Bhave Civil Hospital, Silvassa for their assistance in the laboratory procedures.

Orcid

Ashwin Jawdekar https://orcid.org/0000-0002-9948-9007

References

1. Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet 2007;369(9555):51–59. DOI: 10.1016/S0140-6736(07)60031-2
2. Innes NP, Clarkson JE, Douglas GV, et al. Child caries management: a randomized controlled trial in dental practice. J Dent Res 2020;99(1):36–43. DOI: 10.1177/0022034519888882
3. Gill J. Dental caries: The disease and its clinical management, third edition. Br Dent J 2016;221(8):443–443.
4. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res 2004;38(3):182–191. DOI: 10.1159/000077753
5. Simón-Soro A, Mira A. Solving the etiology of dental caries. Trends Microbiol 2015;23(2):76–82. DOI: 10.1016/j.tim.2014.10.010
6. Krzyściak W, Jurczak A, Kościelniak D, et al. The virulence of Streptococcus mutans and the ability to form biofilms. Eur J Clin Microbiol Infect Dis 2014;33(4):499–515. DOI: 10.1007/s10096-013-1993-7
7. Jothika M, Vanajassun PP, 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(7):44. DOI: 10.4103/2231-0762.156153
8. Lang NP, Hotz P, Graf H, et al. Effects of supervised chlorhexidine mouthrinses in children: a longitudinal clinical trial. J Periodontal Res 1982;17(1):101–111. DOI: 10.1111/j.1600-0765.1982.tb01135.x
9. Walsh T, Jeronimo N, Deborah M. Chlorhexidine treatment for the prevention of dental caries in children and adolescents. Cochrane Database Syst Rev 2015;2015(4):CD008457. DOI: 10.1002/14651858.CD008457.pub2
10. James P, Worthington HV, Parnell C, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Systema Rev 2017;3(3):CD008676. DOI: 10.1002/14651858.CD008676.pub2
11. Karadağ AE, İpekçi E, Yağcılar AP, et al. Antimicrobial activities of mouthwashes obtained from various combinations of Elettaria cardamomum Maton., Lavandula angustifolia Mill. and Salvia triloba L. essential oils. Nat Vol Ess Oils 2020;7(1):9-17. DOI: 10.37929/nveo.685474
12. Erawati S, Rahardjo A, Pintauli S. Clinical efficacy of a new mouthwash containing essential oil of cardamom in reducing volatile sulphur coumpounds concentration. Int J Clin Prev Dent 2014;10(4):237–242.
13. Barroso H, Ramalhete R, Domingues A, et al. Inhibitory activity of a green and black tea blend on Streptococcus mutans. J Oral Microbiol 2018;10(1):1481322. DOI: 10.1080/20002297.2018.1481322
14. Saini R, Saini S, Sharma S. Biofilm: a dental microbial infection. J Nat Sci Biol Med 2011;2(1):71–75. DOI: 10.4103/0976-9668.82317
15. Marsh PD. Microbiology of dental plaque biofilms and their role in oral health and caries. Dent Clin North Am 2010;54(3):441–454. DOI: 10.1016/j.cden.2010.03.002
16. Horst JA, Tanzer JM, Milgrom PM. Fluorides and other preventive strategies for tooth decay. Dent Clin North Am 2018;62(2):207–234. DOI: 10.1016/j.cden.2017.11.003
17. Keijser JA, Verkade H, Timmerman MF, et al. Comparison of 2 commercially available chlorhexidine mouthrinses. J Periodontol 2003;74(2):214–218. DOI: 10.1902/jop.2003.74.2.214
18. de Souza LB, de Aquino SG, de Souza PPC, et al. Cytotoxic effects of different concentrations of chlorhexidine. Am J Dent 2007;20(6):400–404.
19. Goyal AK, Bhat M, Sharma M, et al. Effect of green tea mouth rinse on Streptococcus mutans in plaque and saliva in children: an in vivo study. J Indian Soc Pedod Prev Dent 2017;35(1):41–46. DOI: 10.4103/0970-4388.199227
20. Neturi RS, R S, B VS, et al. Effects of green tea on Streptococcus mutans counts- a randomised control trail. J Clin Diagn Res 2014;8(11):ZC128–ZC130. DOI: 10.7860/JCDR/2014/10963.5211
21. Haydari M, Bardakci AG, Koldsland OC, et al. Comparing the effect of 0.06%-, 0.12% and 0.2% chlorhexidine on plaque, bleeding and side effects in an experimental gingivitis model: a parallel group, double masked randomized clinical trial. BMC oral health 2017;17(1):118. DOI: 10.1186/s12903-017-0400-7
22. Richards D. Chlorhexidine mouthwash plaque levels and gingival health. Evid Based Dent 2017;18(2):37–38. DOI: 10.1038/sj.ebd.6401232
23. Van Strydonck DA, Slot DE, Van der Velden U, et al. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: a systematic review. J Clin Periodontol 2012;39(11):1042–1055. DOI: 10.1111/j.1600-051X.2012.01883.x
24. Anil S, Bhandi SH, Chalisserry EP, et al. Chemical plaque control strategies in the prevention of biofilm-associated oral diseases. J Contemp Dent Pract 2016;17(4):337–343.
25. Vinod KS, Sunil KS, Sethi P, et al. A novel herbal formulation versus chlorhexidine mouthwash in efficacy against oral microflora. J Int Soc Prev Community Dent 2018;8(2):184–190. DOI: 10.4103/jispcd.JISPCD_59_18