Dental Research Journal

Original Article

A comparative evaluation of the antimicrobial effect of chamomile, *Aloe vera*-green tea, and chlorhexidine mouthwashes on some oral bacterial species

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ABSTRACT

Background: Plant compounds such as *Aloe vera*, green tea, and chamomile have been increasingly used in recent years to achieve oral health. This study aimed to determine the antimicrobial effect of chamomile (Matrika), *A. vera*-green tea, and chlorhexidine (CHX) mouthwashes on some oral bacterial species.

Materials and Methods: This prospective experimental study investigated the antimicrobial properties of three mouthwashes, including chamomile (Matrika), *A. vera* -green tea, and CHX as well as distilled water as control on five bacterial species, including *Streptococcus Oralis*, *Streptococcus sanguis*, and *Streptococcus mutans* as primary colonizers and *Porphyromonas gingivalis* and *Eikenella corrodens* as secondary colonizers. Colony-forming unit was used to count the colonies and disc diffusion and well diffusion methods were used to measure the diameter of zone of inhibition. Data were analyzed by SPSS (version 22) software using descriptive statistics, ANOVA, Tukey, Kruskal–Wallis, and Mann–Whitney tests ($\alpha = 0.05$).

Results: CHX had a significantly higher antibacterial effect than the other two mouthwashes in all three methods ($P < 0.001$). Further, the herbal mouthwashes in all three methods had a statistically significant effect on the bacterial species ($P < 0.001$). *A. vera*-green tea mouthwash had a significantly higher effect than chamomile mouthwash (Matrika) on all bacterial species except for *S. sanguis* ($P < 0.05$).

Conclusion: The findings showed that herbal mouthwashes had potentially antibacterial effects, but these effects were significantly lower than that of CHX. However, more clinical studies are needed to prove the current findings.

Key Words: *Aloe vera*, chamomile, chlorhexidine, green tea, mouthwash

Received: 10-Oct-2022
Revised: 10-May-2023
Accepted: 14-May-2023
Published: 27-Jun-2023

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INTRODUCTION

Gingivitis associated with dental plaque is the most common form of gingival disease that leads to destruction of periodontal structure or periodontitis if it is not treated.[1] Formation of dental plaque starts with accumulation of Gram-positive Streptococcus and develops with Gram-negative anaerobic bacterial

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How to cite this article: Naghsh N, Moghareabed A, Nematnejad M, Yaghini J, Sadeghi SM. A comparative evaluation of the antimicrobial effect of chamomile, *Aloe vera*-green tea, and chlorhexidine mouthwashes on some oral bacterial species. Dent Res J 2023;20:70.

colonies.[2] Oral cavity hosts a significant number of Gram-negative and Gram-positive bacteria.[3] The endogenous bacterial species of oral cavity include *Actinobacillus actinomycetemcomitans*, *Fusobacteria*, *Porphyromonas gingivalis*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Bacteroides*, and *Prevotella*, whose metabolites play a major role in the inhibition and development of oral cavity infections.[4,5]

Effective prevention of oral infections is accomplished by mechanical removal of dental plaque via toothbrushing and dental flossing.[6] Despite the significance of mechanical control of dental plaque, gingival inflammation is highly prevalent due to inefficient implementation of these methods. Hence, chemical methods such as use of toothpaste and mouthwashes containing anti-inflammatory and anti-plaque compounds along with mechanical methods are recommended for a proper oral hygiene.[7] Among all available mouthwashes, CHX has a high capacity in reducing dental plaque and pathogenic microorganisms like *S. mutans*.[8] Yet, chlorhexidine (CHX) has side effects such as tooth discoloration, taste change, and mouth drying and burning.[9] Nowadays, scholars have turned to traditional medicine to find better drugs for the treatment of microbial infections.[10] Plant extracts may be used as antibacterial and antifungal agents against common antibiotic-resistant pathogens.[11]

Aloe vera is a member of Aloaceae family[12] that is increasingly being used as an herbal drug for dental problems.[13] *A. vera* gel has inhibitory effects on a number of caries-inducing bacteria and periopathogens.[14] Anthraquinones or phenolic compounds, saponin, tannin, sterol, and organic acids are active agents in *A. vera* gel.[15] Flavonoids such as catechin are bioactive compounds in the leaves of green tea, the most important of which is epigallocatechin gallate.[16] The green tea catechin has bactericidal effects on black pigment bacteria and Gram-negative anaerobic rods, and a combination of mechanical methods and green tea catechin is effective in improving the periodontal condition.[17]

Numerous studies on animal models have shown that green tea has anti-inflammatory, antibacterial, antidiabetic, and most importantly anticancer properties.[18] Chamomile belongs to the Asteraceae family and its pharmaceutical properties are owing to the flavonoid compounds and breakdown of volatile oils. The extracts of this plant have various pharmacological properties such as anti-itching, anti-inflammatory, antimicrobial, antiviral, and antioxidant properties.[15] The main compound of Matrika mouthwash is the extract of chamomile, which inhibits the microbial growth.[19]

Several studies have been done on the herbal products. Abdelmonem *et al*. observed that the number of bacterial colonies of *Porphyromonas gingivalis* and *Prevotella intermedia* significantly decreased in the group receiving *A. vera* mouthwash following SRP treatment.[20] Araghizadeh *et al*. showed the inhibitory effect of green tea on Actinobacillus actinomycetemcomitans, *Prevotella intermedia*, *P. gingivalis*, and *S. mutans* by agar disc diffusion and broth microdilution methods.[21] Hashemipour *et al*. also investigated the antimicrobial effects of CHX mouthwash (Shahr Darou Co.) and chamomile on *Streptococcus sanguis*, *Candida albicans*, *Streptococcus viridans*, *S. mutans*, and *Lactobacillus casei* using serial dilution and reported the higher efficacy of CHX compared to chamomile.[22]

Considering the abovementioned studies and presence of antibacterial agents in *A. vera*, green tea, and chamomile plant extracts, the current study was conducted to determine and compare the antimicrobial effects of chamomile (Matrika), *A. vera*-green tea, and CHX mouthwashes on *S. sanguis*, *S. oralis*, *S. mutans*, *P. gingivalis*, and *Eikenella corrodens* bacterial species.

MATERIALS AND METHODS

This prospective single-blind *in vitro* study investigated the antibacterial effects of Matrika (Barij Essence, Kashan, Iran), *A. vera*-green tea (Barij Essence, Kashan, Iran), and CHX 0.2% (Vi-One, Iran) as well as distilled water as control on five bacterial species, including *S. oralis*, *S. sanguis*, and *S. mutans* as primary colonizers and *P. gingivalis* and *E. corrodens* as secondary colonizers at the Department of Microbiology, School of Medicine, Isfahan University of Medical Sciences, in 2016.

First, some of the suspension containing each bacterium was cultured on the specific culture medium of each species. After incubation, several colonies of each species were separately dissolved in the Trypticase Soy Broth (TSB, BD™, Germany), and a suspension with specific McFarland turbidity was prepared.[23]

In colony-forming unit, 1 cc of each mouthwash and distilled water was separately mixed with 1 cc of the McFarland bacterial suspension and was cultured in a specific culture medium after incubation. Further, the McFarland suspension of each bacterium was cultured on the specific culture medium prior to adjacency. Finally, the number of colonies was counted before and after adjacency.[23]

To analyze the zone of inhibition by disc diffusion method, the McFarland suspension of each bacterial species was cultured on the specific culture medium plate by spread plate technique. Then, the paper discs impregnated with mouthwash or distilled water (20 μL) were placed on the plate and incubated. Finally, the zone of inhibition of each disc was measured.[23]

As for the analysis of zone of inhibition by well diffusion method, the McFarland suspension of each bacterial species was cultured on the specific culture medium plate by spread plate technique. Then, the wells were created on the culture medium and 60 μL of each mouthwash or distilled water was added to the wells and incubated. Finally, the zone of inhibition of each well was measured.[23] In all methods, anaerobic conditions were considered for *P. gingivalis*.

To increase the accuracy of each sample in disc diffusion and well diffusion methods, 15 tests were carried out on each bacterial species. Moreover, five tests were performed for each sample in each bacterial species in colony-forming unit. The obtained data were analyzed by SPSS (version 22, SPSS Inc., Chicago, IL, USA) using descriptive statistics and ANOVA, Tukey, Kruskal–Wallis, and Mann–Whitney tests ($\alpha = 0.05$).

RESULTS

The results of ANOVA showed a significant difference among all the four groups in well diffusion and disc diffusion methods with respect to the mean diameter of zone of inhibition ($P < 0.001$). Further, the mean diameter of zone of inhibition was significantly different among different bacteria in all three mouthwashes ($P < 0.001$) [Tables 1 and 2]. Moreover, the mean diameter of zone of inhibition was equal to 0 in all bacterial species in the control group in both methods.

The findings of Kruskal–Wallis test indicated a significant difference among the four groups in the mean number of the remaining bacterial colonies ($P < 0.001$). Moreover, there was a significant difference among different bacteria in the number of bacterial colonies in both *A. vera*-green tea and chamomile (Matrika) mouthwashes ($P < 0.001$). However, there were no colonies in any of the bacteria in CHX mouthwash [Table 3]. There was also no significant difference between the mean number of

Table 1: Mean and standard deviation of the diameter (mm) of the zone of inhibition in bacterial species by the studied mouthwashes in well diffusion method

Bacteria	Mean±SD			*P*
	CHX	Green tea-*Aloe vera*	Chamomile	
Streptococcus oralis	1.67±0.05	1.19±0.08	0±0	<0.001
Eikenella corrodens	2.03±0.08	0.96±0.06	0.81±0.03	<0.001
Streptococcus sanguis	2.01±0.03	1.04±0.06	1.81±0.05	<0.001
Streptococcus mutans	1.61±0.03	1.05±0.05	0±0	<0.001
Porphyromonas gingivalis	2±0	1.66±0.05	0±0	<0.001
P	<0.001	<0.001	<0.001	-

SD: Standard deviation; CHX: Chlorhexidine

Table 2: Mean and standard deviation of the diameter (mm) of the zone of inhibition in bacterial species by the studied mouthwashes in disc diffusion method

Bacteria	Mean±SD			*P*
	CHX	Green tea-*Aloe vera*	Chamomile	
Streptococcus oralis	1.70±0.05	0±0	0±0	<0.001
Eikenella corrodens	1.80±0.10	0.86±0.05	0±0	<0.001
Streptococcus sanguis	2.02±0.06	0±0	1.65±0.06	<0.001
Streptococcus mutans	1.63±0.07	0.24±0.41	0±0	<0.001
Porphyromonas gingivalis	2±0	1.23±0.26	0±0	<0.001
P	<0.001	<0.001	<0.001	-

SD: Standard deviation; CHX: Chlorhexidine

Table 3: Mean and standard deviation of the number of the remaining colonies of bacterial species by the studied mouthwashes

Bacteria	Mean±SD			*P*
	CHX	Green tea-*Aloe vera*	Chamomile	
Streptococcus oralis	0±0	101±0	1.5×10^{8}±0	<0.001
Eikenella corrodens	0±0	1×10^{3}±0	1.5×10^{8}±0	<0.001
Streptococcus sanguis	0±0	1×10^{8}±0	$2.8\times10^{2}\pm4\times10^{2}$	<0.001
Streptococcus mutans	0±0	1×10^{4}±0	1×10^{8}±0	<0.001
Porphyromonas gingivalis	0±0	1×10^{3}±0	1×10^{8}±0	<0.001
P	1	<0.001	<0.001	-

SD: Standard deviation; CHX: Chlorhexidine the remaining colonies and the primary colonies in all bacterial species in the control group.

DISCUSSION

The results of this study showed that CHX (which is known as the gold standard antibacterial mouthwash) had a significantly higher effect on all five bacterial species than the other two mouthwashes. Further, each mouthwash had a different effect on various bacterial species.

Regarding the comparison of zone of inhibition by disc diffusion and well diffusion methods, the findings indicated no significant difference for the effect of CHX in both methods for each bacterial species except *E. corrodens*. As for herbal mouthwashes in disc diffusion method, no zone of inhibition was observed in most bacterial species and the diameter of zone of inhibition was small. Moreover, the diameter of the zone of inhibition induced by the herbal mouthwashes was higher in well diffusion method than disc diffusion method, and a greater number of bacterial species had no zone of inhibition. The amount of the mouthwash used was higher in well diffusion method than disc diffusion method, but given the similar diameters of the zone of inhibition in each bacterial species in both methods, the difference in CHX mouthwash might probably be due to the difference in the inherent diffusion properties of mouthwashes. It seems that the herbal mouthwashes have not been able to diffuse well from the disc to the culture medium after impregnation of discs in disc diffusion method. In well diffusion method, considering the absence of disc and higher amount of the mouthwash used, the zone of inhibition was formed in a greater number of bacterial species. Moreover, the diameter of the zone of inhibition was larger. Saliem investigated the antibacterial effect of the green tea extract, obtained by alcohol and water, and CHX on *P. gingivalis* using well diffusion and serial microdilution methods and measured the minimum inhibitory concentration and minimum bactericidal concentration. The researcher concluded that ethanolic green tea was more effective than CHX and water-based green tea.[24] It seems that the interaction of mouthwash and bacteria is an important factor in evaluating the effect of herbal mouthwashes on bacterial species. It can be concluded that the findings of colony-forming unit in which the mouthwash and bacteria blend are closer to reality.

The present study showed that CHX mouthwash had the lowest effect on *S. mutans* in both disc diffusion and well diffusion methods, while its effect was equal on all bacterial species in colony-forming unit, and the number of colonies after adjacency with the mouthwash was equal to 0. Research has shown that CHX has a high capacity in reducing the dental plaque and pathogenic microorganisms like *S. mutans* among all mouthwashes available,[8] which is in line with the results of the present study with respect to the colony-forming unit.

In this study, the effect of *A. vera*-green tea mouthwash on *S. mutans*, *P. gingivalis*, and *E. corrodens* was higher than that of Matrika mouthwash in all three methods. However, its effect on *S. sanguis* was lower than that of Matrika in all three methods, but it showed a significant difference compared to the control group (distilled water). Further, the effect of this mouthwash on *Streptococcus oralis* was higher than that of Matrika mouthwash in colony-forming unit and well diffusion method.

Sakanaka *et al*. showed that green tea catechin had inhibitory effects on *S. mutans*. However, the efficacy of green tea and CHX was not compared in this study.[25] Araghizadeh *et al*. reported a higher inhibitory effect of green tea on *S. mutans* than periopathogenic bacteria. It was confirmed that the sensitivity of Gram-positive bacteria might be due to the difference in the cell wall and bacterial polysaccharide charge.[21]

Hirasawa *et al*. indicated that green tea catechin had bactericidal effects on the black pigment bacteria and Gram-negative anaerobic rods, and a combination of mechanical methods and green tea catechin improved the periodontal conditions.[17] In the current study, *A. vera*-green tea had the highest effect on *P. gingivalis* and the greatest diameter of zone of inhibition was 17 mm. The results of the present study also showed that the effect of *A. vera*-green tea on *S. sanguis* was much lower than those of other bacterial species in colony-firming unit. Since this bacterium is similar to *S. mutans* and Gram-positive *S. oralis* cocci, this difference may be due to the intrinsic properties of each species.

Using microdilution and disc diffusion methods, Fani and Kohantalab found that *A. vera* gel had inhibitory effects on some caries-inducing and periopathogenic bacteria. They concluded that a proper concentration of *A. vera* gel could be used in toothpaste or mouthwash to prevent caries and periodontal diseases.[14] The findings of Lee *et al*. showed that *A. vera* toothpaste had inhibitory effects on the growth of different oral microorganisms such as *S. mutans* and *S. sanguis*,[26] which is in agreement with the results of the present study. Bertolini *et al*. also found no significant difference between the antimicrobial effects of *A. vera*-beeswax and CHX gluconate 0.12% mouthwashes on *S. mutans* in colony-forming unit.[27] However, our study showed that CHX had a significantly higher effect on the studied bacterial species than *A. vera*-green tea mouthwash in all methods.

Sadeghi reported a significantly higher effect of CHX on *S. mutans*, *S. sanguis*, and *E. corrodens* than chamomile mouthwash using disc diffusion method.[19] The findings of the study by Salehi *et al*. on orthodontic patients indicated a significant reduction of microorganisms during the use of Matrika and Persica mouthwashes.[28]

In the present study, CHX mouthwash exhibited a significantly higher effect than Matrika on all five bacterial species in all methods. Moreover, Matrika mouthwash showed a significant effect on *S. mutans*, *S. sanguis*, and *P. gingivalis* in colony-forming unit but had no effect on *E. corrodens* and *S. oralis*. In addition, Matrika had the highest effect on *S. sanguis*. Since this bacterium is similar to *S. mutans* and Gram-positive *S. oralis* cocci, this difference might be due to the inherent properties of the bacterial species.

The findings of Hashemipour *et al*. also showed that CHX had a higher effect than chamomile on *Lactobacillus casei*, *C. albicans*, *S. sanguis*, *S. viridans*, and *S. mutans* in serial dilution method,[22] which is in line with the results of the current study.

A limitation of this study was lack of access to other species like periopathogens. Moreover, the mouthwashes used in this study were made beforehand and it was not possible to change their concentration. In addition, given the different growth conditions of oral bacterial species compared to *in vitro* conditions, further clinical studies are suggested to explore the effect of herbal mouthwashes. Furthermore, the adjacency of plant compounds and microorganisms is suggested to be taken into account in analyzing the antimicrobial effects of plant compounds.

CONCLUSION

The findings of the present study showed that *A. vera*-green tea and Matrika mouthwashes had potentially antibacterial effects, but these effects were lower than that of CHX mouthwash. Within the limitations of this study, it seems that these herbal mouthwashes cannot be an appropriate substitute to CHX mouthwash. However, more clinical studies are needed to prove the present findings.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments

We would like to thank all of participants who help us to conducting this study. Based on a thesis submitted to the school of Dentistry, Isfahan University of Medical Sciences, in partial fulfillment of the requirement for the DDS/MSC degree, this study was supported by Isfahan University of Medical Sciences Research (Grant No. 396279).

Financial support and sponsorship

This study has been supported by the Isfahan University of Medical Sciences.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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