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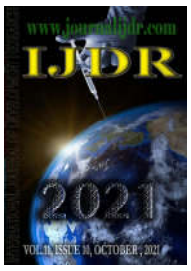

RESEARCH ARTICLE OPEN ACCESS

ANTIMICROBIAL AND ANTIBIOFILM OF ALOE VERA ON BACTERIA

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ABSTRACT

Objective: This study aimed to evaluate the antimicrobial and antibiofilm potential of 10% *Aloe vera* gel against *in vitro* oral bacteria compared with 2% Chlorhexidine Gluconate gel. **Design:** The antimicrobial activity was evaluated using radial diffusion and agar perforation. The results were identified by the presence and visual size of the halo of inhibition of the bacterial growth around the holes. The microbial biofilm formation assay was conducted in 24-well surface-modified polystyrene plates with flat bottom. All experiments were performed in triplicate with the respective results categorized and further statistical analysis in software GraphPad Prism. For the analysis of intergroup differences, ANOVA with Bonferroni post-test and significance level of 1% were performed. **Results:** The 10%*Aloe vera* gel presented antimicrobial activity against all the oral bacteria analyzed and such activity was greater than that of the 2% Chlorhexidine Gluconate gel against some strains. The antibiofilm potential did not significantly affect the oral bacteria analyzed, with similar results observed for all the strains. **Conclusions:** The elucidation and analysis of the results of the present study demonstrated the antimicrobial activity of the 10% *Aloe vera* gel against all the oral bacteria analyzed. However, the antibiofilm potential of the 10% *Aloe vera* gel did not significantly affect the oral bacteria tested.

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INTRODUCTION

Socransky and Haffajee (2005) reported that periodontal diseases and dental caries are the most prevalent oral infections that affecthumanity around the world. Oral bacterial species such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Bacteroides sp., Prevotella sp., Fusobacterium sp.* - related to periodontal disease; and *Streptococcus oralis, Streptococcus parasanguinis, Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguinis, Streptococcus sobrinus, Lactobacillus acidophilus and Lactobacillus rhamnosus* – to dental caries – playimportant roles, along with their active metabolites, in initiating and progressing these infections. The bacterial biofilm is the main etiological factor in gingivitis and periodontitis and action is needed to maintain periodontal health and/or prevent disease progression by removing supragingival and subgingival bacterial biofilms (Donlan & Costerton, 2002). Different from planktonic cells, organized bacteria – such as biofilms – are substantially resistant to antibiotics and other antibacterial compounds (Obst, Schwartz and Volkmann, 2006). The chlorhexidine is currently the most effective antiplaque and anti-gingivitis agent available and is generally accepted as a gold standard, it has been shown to be inactivated by blood and serum (Eick, Radakovic, Pfister, Nietzsche and Sculean, 2012; Oosterwaal, Mikx, van den Brink and Renggli, 1989). Thus, the effects of chlorhexidine observed in the supragingival environment cannot simply be extrapolated to the subgingival environment (Arweiler, Auschill and Sculean, 2018). In addition, Parekh and Chanda (2007) reported some side effects, such as changes in taste, staining of teeth, restorative material and dorsum of the tongue, and formation of supragingival calculus. Herbal medicines have been used since ancient times in the treatment of various diseases, including periodontitis (Cragg and Newman, 2013). *Aloe vera* is a cactus plant of the *Liliaceae* family with about 360 species containing 75 active ingredients such as vitamins, enzymes, minerals, sugars, saponins, salicylic acids and amino acids. It has been used as anti-inflammatory and antibacterial (Voglerand and Ernst, 1999). A study conducted by Patri and Sahu (2017) compared the efficacy of plant-derived antibacterial agents – including 1% Tea Tree oil and *Aloe vera* gel without reporting the concentration used – with 2% Chlorhexidine as cavity disinfectant for use in minimally invasive dentistry. The study concluded that removal of carious tissue alone does not eliminate all cariogenic bacteria. Natural antibacterial agents with published evidence of their efficacy can be effectively used as dental cavity disinfectants, thus minimizing secondary caries and promoting long-term restorative success. More recently, a study conducted by Moghaddam, Radafshar, Jahandideh and Kakaei (2017) with humans with chronic

periodontitis undergoing non-surgical treatment demonstrated that scaling and root planing combined with adjuvant 98% *Aloe vera* gel therapy resulted in significant improvements in severe periodontitis. Thus, the present *in vitro* study aimed to assess the antimicrobial and antibiofilm effects of 10% *Aloe vera* gel on oral bacteria.

MATERIALS AND METHODS

Study Design: Laboratory, microbiological, interventional and cross-sectional study.

Proposed Methodology

Obtaining test substances: The formulations of 10% *Aloe vera* gel and 2% Chlorhexidine Gluconate gel were obtained by manipulation in a specialized pharmacy. All chemical agents used were gel, used in their respective methodologies. Strains used and growing conditions. The bacterial strains *Streptococcus mutans* ATCC25175, *Streptococcus oralis* ATCC10557, *Streptococcus salivarius* ATCC7073, *Streptococcus parasanguinis* ATCC903, *Streptococcus sanguis* ATCC10556, *Lactobacillus acidophilus* ATCC4356, *Lactobacillus rhamnosus* ATCC7469 and *Porphyromonas gingivalis* ATCC33277 were grown in media suitable for their ideal growth conditions. All the strains were grown individually after initial inoculation in 5mL of sterile medium for 24h at 37°C and microaerophilia for all *Streptococcus* and *Lactobacillus* and anaerobiosis for *Porphyromonas gingivalis*. After that, a new aliquot was removed and inoculated into 5 ml of sterile supplement under the same conditions. However, in this second phase, the bacterial cells that presented exponential growth according to the previously measured growth curve were diluted to concentrations of 1 X 10^6 $CFU.mL^{-1}$ in BHI supplemented for the antimicrobial activity experiments and of 2 X 10^7 $CFU.mL^{-1}$ for antibiofilm activity experiments.

Evaluation of antimicrobial activity: The tests with the different gels were performed using Petri plates containing BHI agar medium supplemented with 1% sucrose for growing *Streptococcus* and *Lactobacillus* and blood agar supplemented with Methionine for growing *Porphyromonas gingivalis*. Holes were drilled in each plate using 5 mm diameter cylinders to form wells in which it was possible to add the substances that should be analyzed. The wells were filled with 20µL of 10% *Aloe vera* gel according to the technique described by Moody, Adebiyi and Adeniyi (2004). Each of the following gels were tested: Carbopol gel (Negative Control), 2% Chlorhexidine Gluconate gel (Positive Control) and 10% *Aloe vera* gel (Test substance). A sterile swab was used for the distribution of the bacterial suspension in Agar. The excess was withdrawn by applying light pressure on the wall of the tube and then the swab was smoothly inoculated onto the surface of the plate with movements in three different directions. A new swab was used for each plate. After preparation and pouring, the plates were incubated in an oven under ideal atmosphere conditions (5% CO_2 for *Streptococcus* and *Lactobacillus*; anaerobiosis for *Porphyromonas gingivalis*) at 37°C for 24h for *Streptococcus* and *Lactobacillus* and 7 days for *Porphyromonas gingivalis*. The microbial activity was evaluated by the classical methods of radial diffusion and agar plate perforation and the results were identified by the presence and visual size of the halo of inhibition of the microbial growth around the holes. Three measurements were performed, one for each perforation in each group, using a millimeter ruler. We calculated the mean of the halo of inhibition per group. The measurements were performed by a single examiner.

Evaluation of antibiofilm activity: The microbial biofilm formation assay was performed according to the methodology described by O'toole and Kolter (1998) on 24-well surface-modified polystyrene plates with flat bottom. First, 15 wells were filled with 1mL of bacterial solution at the concentration of 2 X 10^7 $CFU.mL^{-1}$ and incubated in a bacteriological oven under the same conditions previously described for 24 hours for *Streptococcus* and *Lactobacillus* and 7 days for *Porphyromonasgingivalis*. After the growth period, the supernatant was gently removed from each well and the surface of each well was washed 3 times with sterile 0.9% NaCl solution in order to remove the cells poorly adhered to the biofilm. After that, 1mL of the following substances were added to 5 wells: 10% *Aloe vera* Gel (Test Substance), 2% Chlorhexidine Gluconate Gel (Positive Control) and Sterile Culture Medium (Negative Control). Each substance acted for 5 minutes and was subsequently removed from each well. After that, the wells were washed 3 times with sterile 0.9% NaCl solution. After washing, 200mL of methyl alcohol P.A was added to fix the adhered cells. After removal of the methanol, 200mL of 0.1% crystal violet was added for 15minutes. This process was performed to allow indirect quantification of biofilm formation through crystal violet staining. After that, the washing process was repeated, and the plate was put at room temperature for 1 hour for drying. 200 mL of 33% acetic acid was added for 15 minutes for the dissolution of the dye bound to the biofilm. The obtained suspension was transferred to another sterile 96 well plate and absorbance in each well wasmeasured using a spectrophotometer (Sunrise™Absorbance Reader from TecanTrading AG, Männedorf, Switzerland) at 590 nm.

Another plate submitted to the same conditions described above was incubated for 24h at 37°C and then washed three times with sterile distilled water to remove poorly adhered cells. After that, 200mL of sterile 0.9% NaCl solution was added to each well in the plate which was then submitted to ultrasonic bath (SC-52 from Sonicator, Newtown, Connecticut, EUA) for 6 minutes to release the biofilm-forming cells. The volume of each well was removed and pooled in a sterile falcon tube – the final volume was 3mL. A new 96 well plate was used for a 10-fold dilution of the cell suspension for further plating. The plates were then incubated at 37°C for 24h. After counting the number of colonies grown on each plate, the number of cells was corrected by multiplying the number of Colony Forming Units (CFU) on the plate after dilution. The value was expressed as $CFU.mL^{-1}$.

Statistical analysis: All experiments were performed in triplicate with the respective results categorized in Microsoft Excel (Version 2012 for Windows- Redmond, Washington, USA) and further statistical analysis in software GraphPad Prism5(GraphPad Software Inc.– San Diego, California, USA). For the analysis of intergroup differences, ANOVA with Bonferroni post-test and significance level of 1% were performed.

RESULTS

Antimicrobial Activity Assays: Figure 01 shows the antimicrobial activity of the 10% *Aloe vera* gel compared with the Positive Control (2% Chlorhexidine Gluconate Gel). The 10% *Aloe vera* gel presented antimicrobial activity against all strains analyzed. It should be noted that the antimicrobial activity against the strains of S. *parasanguinis* and *L. rhamnosus* wasgreater than thatpresentedby the positive control. As for the other strains, the values were lower or similar to those obtained with 2% Chlorhexidine Gluconate.

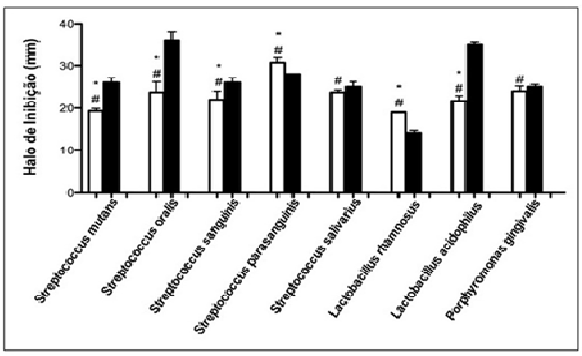

Figure 1: Antimicrobial activity of the 10% *Aloe vera* gel against the different strains tested. 10% *Aloe vera*gel (□), Carbopol gel (▩) and 2% Chlorhexidine Gluconate gel(■).* p < 0.05 for Negative Control (Carbopol gel) and #p < 0.05 referring to Positive Control (2% Chlorhexidine Gluconate gel).

Antibiofilm Activity Assays: To analyze the effect of the 10% *Aloe vera* gel on mature monospecies biofilm we used two methodologies for each time of attack with the antimicrobial agent.The quantification of biomass immediately after the attack with the different antimicrobial agents for 5 minutes (Figure 02). There was no statistically significant difference between the 10% *Aloe vera* and the Positive Control. However, the number of CFU immediately after 5 minutes of contact of the antimicrobial agents with the biofilm was significantly lower compared with the Negative Control. This reduction occurred in both the test and positive control groups and there were no statistically significant differences between them in relation to all the bacterial strains analyzed (Figure 03). A new quantification of biomass was performed 24h after the 5minute contact ofthe antimicrobial agents with the mature biofilm (Figure 04). There was a significant reduction in total biomass in all the bacterial strains analyzed.

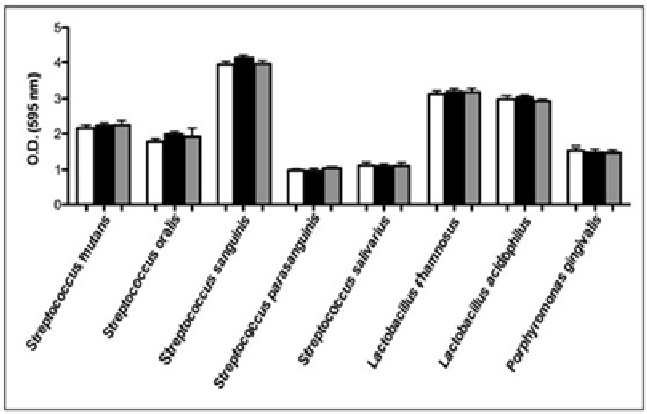

Figure 2: Antibiofilm activity of the 10% *Aloe vera* gelagainst the different strains tested. Verification through quantification of biomass by instant violet crystal after application of agents in mature biofilm. 10% *Aloe vera* gel (□), Carbopol gel (▣) and 2% Chlorhexidine Gluconate gel (■). * p < 0.05 for Negative Control (Sterile Culture Medium) and #p < 0.05 referring to Positive Control (2% Chlorhexidine Gluconate gel)

This was probably due to cell death in the period of contact with antimicrobial agents. It should be noted that there were no differences between the test and positive control groups.

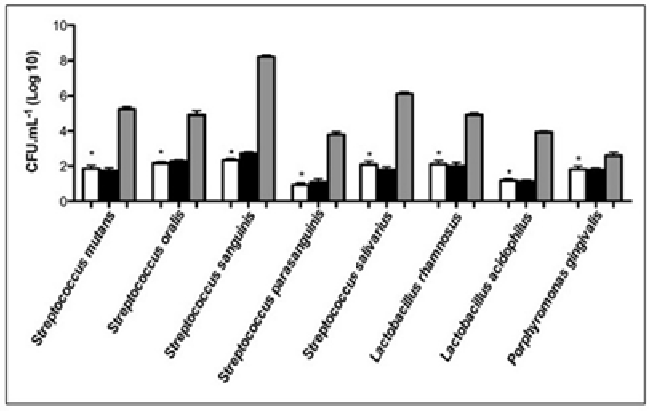

Figure 3: Antibiofilm activity of the 10% *Aloe vera* gel against the different strains tested.Verification by counting of immediate ColonyForming Units (CFU) after application of agents in mature biofilm. 10% *Aloe vera* gel (□), Carbopol gel (▣) and 2% Chlorhexidine Gluconate gel (■). * p < 0.05 for negative control (Sterile Culture Medium) and #p < 0.05 referring to Positive Control (2% Chlorhexidine Gluconate gel)

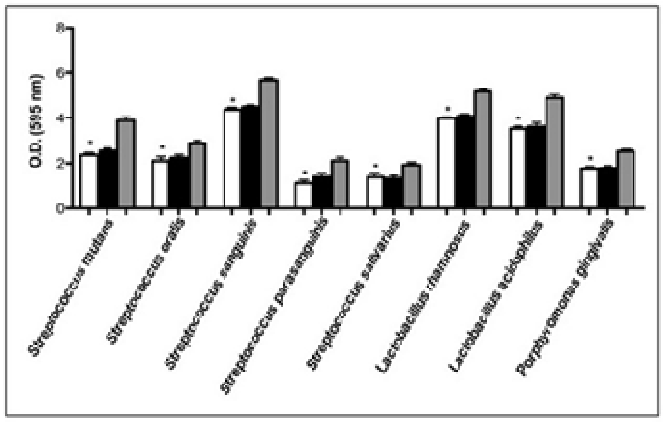

Figure 4: Antibiofilm activity of the 10% *Aloe vera* gel against the different strains tested.Verification by quantification of biomass by violet crystal 24 h after application of agents in mature biofilm. 10% *Aloe vera* gel (□), Carbopol gel (▣) and 2% Chlorhexidine Gluconate gel (■). * p < 0.05 for Negative Control (Sterile Culture Medium) and #p < 0.05 referring to Positive Control (2% Chlorhexidine Gluconate gel).

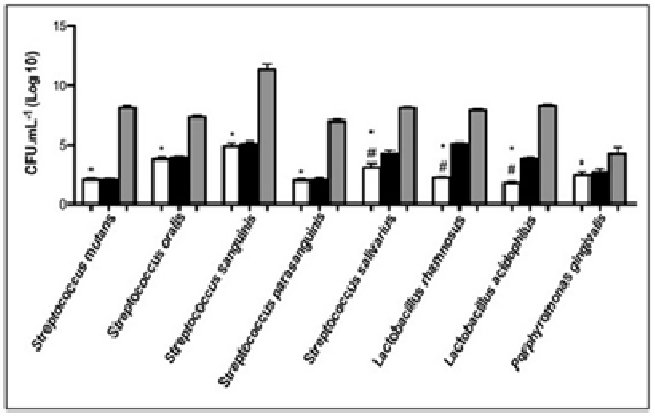

Figure 5. Antibiofilm activity of the 10% *Aloe vera* gel against the different strains tested.Verification by counting of immediate ColonyForming Units (CFU) 24 h after application of agents in mature biofilm. 10% *Aloe vera* gel (□), Carbopol gel (▣) and 2% Chlorhexidine Gluconate gel (■). * p < 0.05 for Negative Control (Sterile Culture Medium) and #p < 0.05 referring to Positive Control (2% Chlorhexidine Gluconate gel)

The number of CFU of the mature biofilm grown under the same conditions (Figure 05). All the biofilms exhibited a reduction in the number of viable cells inside them. However, for strains of *S. salivarius*, *L. rhamnosus* and *L. acidophilus*, the 10% *Aloe vera* gel exerted an activity superior to that of the 2% Chlorhexidine Gluconate gel, but without a reduction in the amount of biomass. This finding may be related to the up-regulation of genes involved in cell stress, which leads to biomass maintenance despite the reduction in the number of viable cell.

DISCUSSION

Oral diseases are among the major public health problems and the most common chronic diseases affecting humanity. The use of natural products for the control of oral diseases is an interesting alternative to synthetic antimicrobials due to its less negative impact on people's health and the effort to overcome primary or secondary resistance to the drug during treatment (Chandra Shekar, Nagarajappa, Suma and Thakur, 2015). The need for new antibiotics and the antimicrobial resistance in oral biofilms has led to a growing interest in the potential of medicinal plants in the treatment of oral diseases.

In the last decade there was an increase in the number of *in vitro* and *in vivo* studies of herbs traditionally used in ethnopharmacological applications due to their antibacterial properties (Milovanova-Palmer and Pendy, 2018). The polysaccharides of the *Aloe vera* gel play a direct role in its bacterial activity by stimulating phagocytic leucocytes that destroy bacteria (Pugh, Ross, ElSohly and Pasco, 2001). *Aloe vera* has been described as an antibacterial agent (Pandey and Mishra, 2010). Anthraquinones are an active compound present in *Aloe vera* and are structurally analogous to tetracycline; therefore, they inhibit bacterialprotein synthesis by blocking the A site on the ribosome (where the RNAt amino acid is inserted) (Habeeb et al., 2007). The present study is the first to evaluate the antibiofilm activity on the bacteria *Lactobaccilus rhamnosus*, *Lactobaccilus acidophilus* and *Porphyromonas gingivalis*. Therefore, the findings cannot be compared with other studies. Pandey and Mishra(2010)assessed the susceptibility of Gram-positive and Gram-negative bacteria to an extract of the *Aloe vera* gel at different concentrations not specified in the study in their respective solvents (ethanol and MiliQ water) to determine the minimum inhibitory concentration (MIC). They found that the ethanol extract of *Aloe vera* leaf had high antibacterial activity against Gram-negative and Gram-positive bacteria with a very low MIC. Our findings agree with the findings reported in the study by Pandey and Mishra(2010) in which a tested product also originated from the same herbal medicine exerted antimicrobial action against Gram-positive and Gram-negative strains. However, the study used a different type of gel and a different methodology. Fani and Kohanteb (2012) inhibited protein synthesis in clinically isolated cariogenic and periodontopathogenic bacterial cells. The antibacterial activity of *Aloe vera* gel – concentration not reported – was determined by the standard disk diffusion sensitivity test in solid media. Undiluted *Aloe vera* gel produced significant growth inhibition zones against all the oral bacteria tested. The diameter of the growth inhibition zone was directly proportional to the concentration of the *Aloe vera* gel. The zone of inhibition produced by undiluted *Aloe vera* gel was wider for *S. mutans* (54 mm) and narrower for *P. gingivalis* (32 mm). At a dilution of 1:8 (12.5%) the *Aloe vera* gel inhibited only *S. mutans* with a zone of inhibition of 10 mm and all isolates of *A. actinomycetemcomitans*, *P. gingivalis* and *B. fragilis* were resistant to this dilution.

In our study, antimicrobial activity was exerted against all the strains analyzed, but there were differences in the results regarding the antimicrobial activity of *Aloe vera* gel against *Streptococcus mutans*. The antimicrobial activity of 2% Chlorhexidine Gluconate was greater than that of the test substance (10% *Aloe vera* gel). The microbial activity of *Porphyromonas gingivalis* was similar for both the test substance and 2% Chlorhexidine Gluconate. Da Silva et al. (2013) evaluated the antimicrobial activity of the synthetic peptide Lys-a1 on the planktonic and biofilm growth of bacteria. The methods for evaluating antimicrobial activity included: MIC and Minimum Bactericidal Concentration (MBC) and quantification of biomass and counting of CFU for biofilm growth. 0.12% Chlorhexidine Gluconate solution was used as a positive control and the BHI culture medium was used as a negative control. The peptide tested showed a remarkable antimicrobial effect and inhibited planktonic and biofilm growth of all the strains tested even at low concentrations. *Streptococcus salivarius* and *Streptococcus parasanguinis* were clearly more susceptible to Lys-a1, and *Streptococcus mutans* exhibited remarkable resistance. The MBC values were generally 1 to 2 times higher than the MIC values in all the strains. Although the methodology used in our study was different from that used by Da Silva et al.(2013) and we evaluated the antimicrobial activity of different substances, the results were similar for some of the microorganisms studied. *Streptococcus mutans* also exhibited remarkable resistance to the test substance (10% *Aloe vera* gel). *Streptococcus parasanguinis* presented opposite results with regard to antimicrobial activity. It was more susceptible to the test substance (10% *Aloe vera* gel) compared with the 2% Chlorhexidine Gluconate gel. The test substance (10% *Aloe vera* gel) presented a slightly inferior antimicrobial activity against *Streptococcus salivarius*, which can be compared to the even more extensive inferiority presented in the results of Da Silva et al.(2013) with the use of the synthetic peptide Lys-a1. The results obtained for *Streptococcus oralis* and *sanguinis* also corroborated the results reported by Da Silva et al. (2013) – these strains were susceptible to the antimicrobial activity of the synthetic peptide Lys-a1 – as their microbial activity was lower with the use of 2% Chlorhexidine Gluconate gel. Evaristo et al.(2014) evaluated the antimicrobial effect of 3β, 6β, 16β-trihydroxylup 20 (29)-ene (CLF1), a triterpene isolated from *Combretum leprosum Mart*., on inhibition of planktonic and biofilm growth of Gram-positive bacteria: *Streptococcus mutans* and *S. mitis*. The antimicrobial activity was evaluated by determining MIC and MBC. MIC showed a complete inhibition of visible bacterial growth. Both [EECL (ethanolic extract of *C. leprosum*) and CLF1] inhibited the growth of *Streptococcus mutans* and *S. mitis*, but CLF1 presented lower MIC and MBC values compared with EECL. On the other hand, the same effect was not observed for Gram-negative *Pseudomonas aeruginosa* and *Klebsiella oxytoca*. When compared with EECL, CLF1 inhibited the growth of *Streptococcus mutans* more than 16 times. In the present study, besides the methodological differences in relation to the study by Evaristo et al.(2014) and the evaluation of the antimicrobial activity of different substances, it was possible to observe a total discrepancy in the antimicrobial activity against *Streptococcus mutans* achieved by 2% Chlorhexidine Gluconate compared withthe same inhibition achieved by triterpene CLF1 in the study by Evaristo et al.(2014). Jain et al.(2015) conducted a study with 45 children aged 3-15 years for the acquisition of clinical strains of *Streptococcus mutans* obtained using the Dentocult® SM Strip Mutans Kit (Orion Diagnostica Oy, Espoo, Finland) instructions.

The researchers observed the comparative evolution of the antibacterial activity of six extracts of Indian plants (including *Aloe vera*) and 0.2% Chlorhexidine against the clinical strains of *Streptococcus mutans* isolated from plaque samples. The six plant extracts were prepared in three different forms: aqueous extracts, organic solvent-based extracts and crude (raw) extracts. The antimicrobial activity of the extracts was determined by measuring the mean zones of inhibition (mm) produced against the bacterial isolates. The results showed that 0.2% chlorhexidine exhibited the maximum antibacterial efficacy against *Streptococcus mutans* as a positive control. Maximum inhibitory activity was achieved by the garlic and the minimum inhibitory activity was achieved by the amla. *Aloe vera* exhibited resistance in the raw form and susceptibility in the forms of organic solvents and aqueous extract. Ginger, neem, and tulsi did not relatively achieve inhibition in their crude forms. Unlike the study by Jain et al. (2015), our study did not obtain successful antimicrobial activity against *Streptococcus mutans* using the 10% *Aloe vera* gel compared with 2%Chlorhexidine Gluconate gel. It should be noted that both studies used products in different compositions and presentations and also different methodologies. The study Da Silva et al.(2017) described the synthesis of a new synthetic peptide based on the primary structure of the peptide KR-12 and evaluated its antimicrobial activity against *Streptococcus mutans*. The antimicrobial effect of KR-12 and [W7] KR12-KAEK was evaluated by determining the MIC. 0.12% Chlorhexidine Gluconate and BHI medium were used as positive and negative controls, respectively. Both peptides showed considerably different activities against the strains tested. While KR12 showed no significant antimicrobial activity, peptide [W7] KR12-KAEK presented bacteriostatic effects. The present study evaluated the antimicrobial activity of different substances and used different methodologies compared with the study by Da Silva et al.(2017). There were differences in antimicrobial activity against *Streptococcus mutans*. The test substance (10% *Aloe vera* gel) showed significant antimicrobial activity but did not obtain a similar or superior performance compared with 2% Chlorhexidine Gluconate.

In the study by Da Silva et al. (2013), the peptide Lys-a1 effectively inhibited biofilm growth for 24h. Data analysis showed a significant difference ($p<0.001$) compared with the negative control (BHI culture medium). The peptide Lys-a1 could inhibit biofilm formation in all the bacterial species tested (*Streptococcus mutans*, *Streptococcus oralis*, *Streptococcus sanguinis*, *Streptococcus parasanguinis*,

Streptococcus salivarius and S*treptococcus sobrinus*). Microbial biofilm was reduced by 98-100% for *S. oralis*, *S. sanguinis* and *S. sobrinus* strains, but there was no significant difference in relation to the 0.12% Chlorhexidine Gluconate solution. *S. parasanguinis* was more susceptible to Lys-a1 and biofilm formation was reduced by more than 70%. A similar result was also observed for *S. salivarius*. *S. mutans* was more resistant to biofilm inhibition using the peptide Lys-a1. At higher concentrations, it interfered significantly in biofilm formation, with biomass reduction of 10 to 88%, which could cause biomass reduction of 99%. There were no differences in relation to 0.12% chlorhexidine gluconate solution. Although the methodology used in our study was similar to that used in the study by Da Silva et al.(2013), there was no statistically significant difference between 10% *Aloe vera* gel and 2% Chlorhexidine Gluconate gel after quantification of biomass immediately after the attack with the different microbial agents for 5 minutes. But 24h after the 5minute contact of the antimicrobial agents with the mature biofilm, the new quantification of biomass showed a significant reduction in total biomass for all the bacterial strains tested. This was probably due to cell death during the period of contact with antimicrobial agents. In the study by Da Silva et al.(2013), CFU were counted only at concentrations below the MBC because there were no colonies to count at higher concentrations due to the bactericidal activity of the peptide. The peptide Lys-a1 exhibited dose-dependent activity against all the tested microbial species. *S. parasanguinis* showed the highest susceptibility to the peptide and it significantly reduced the number of viable bacterial cells at low concentrations. Although *S. mutans* was resistant to Lys-a1, the number of viable cells in the biofilm was reduced. The peptide reduced the number of cells by approximately 60% at lower concentrations and achieved a reduction of more than 90% at higher concentrations.

Although our study used similar methodology, the number of CFU immediately after 5 minutes of contact of the antimicrobial agents with the biofilm was significantly lower in comparison with the Negative Control (Sterile Culture Medium). This reduction was observed for both the test group and the 2% chlorhexidine gluconate group, with no statistically significant difference between the two groups for all the bacterial strains tested. It is not possible to compare our findings with those reported in the study by Da Silva et al. (2013) due to the difference in the time of CFU counting, which occurred immediately after the contact of the antimicrobial agents with the biofilm. Our study and the study by Da Silva et al.(2013)used similar methodology and time to count the CFU of the mature biofilm cultured under the same conditions. We found that all the biofilms presented a reduction in the number of viable cells inside them. However, 10% *Aloe vera* gel exhibited better activity against *Streptococcus salivarius* and *Lactobacillus acidophilus* strains compared with 2% Chlorhexidine Gluconate gel, but without a reduction in the amount of biomass. *Streptococcus mutans* – as in the study by Da Silva et al. (2013) – was also resistant to test substance, but the performance of 2% Chlorhexidine Gluconate gel was the same. The evaluation of antibiofilm activity in the study by Evaristo et al.(2014) showed that CLF1 was only effective in biofilms of Gram-positive strains. CLF1 inhibited *S. mutans* and *S. mitis* biomass by 97% and 90%, respectively. As for the ethanolic extract of *C. leprosum* (EECL), the inhibition was approximately 97.3% for *S. mutans* and 44% for *S. mitis*. According to these findings, the efficiency of CLF1 is very similar to that of chlorhexidine at 125 and 31.25 µg/mL for both bacteria. The results showed that CLF1 decreased the viability of bacterial cells. In the present study, the quantification of biomass immediately after the attack with the different antimicrobial agents for 5 minutes did not differ significantly between 10% *Aloe vera* gel group and the 2% Chlorhexidine Gluconate gel group. These findings cannot be compared with the study by Evaristo et al. (2014) as it did not evaluate the ability of CLF1 to quantify biomass immediately after the attack. It is possible to compare biomass quantification 24h after application of the agents in the mature biofilm in the present study with that in the study by Evaristo et al.(2014) despite the differences in methodology and antimicrobial products used. There was a significant reduction in total biomass for all the bacterial strains tested. It should be noted that there was no difference between the test group and the 2% Chlorhexidine Gluconate gel group. Therefore, *Streptococcus mutans*, as Gram-positive, presented similar biomass quantification for both the test substance and the 2% Chlorhexidine Gluconate gel, thus corroborating the experiment of Evaristo et al.(2014)using CLF1. In contrast with the findings of Evaristo et al.(2014), who reported only an inhibition of biomass in Gram-positive bacteria (*Streptococcus mutans* and *Streptococcus mitis*) with the use of CLF1, the substance used in present study could inhibit the biomass in Gram negative strains (*Porphyromonas gingivalis*). In the study by Da Silva et al.(2017), treatment with KR12 did not result in significant changes in biofilm formed by *S. mutans*. On the other hand, the peptide [W7] KR12-KAEK inhibited the production of biomass by all the strains at the maximum concentrations tested. The quantification of biomass immediately after the attack with the different antimicrobial agents for 5 minutes cannot be compared with the study by Da Silva et al.(2017) as it did not evaluate the ability of KR12 to quantify biomass immediately after the attack. It was possible to compare biomass quantification 24h after application of the agents in the mature biofilm in the present study with that in the study by Da Silva et al. (2017) despite the similar methodology and the different antimicrobial product (peptide KR12). There was a significant reduction in total biomass for all the bacterial strains tested. There was no difference between the test group and 2% Chlorhexidine Gluconate gel. Therefore, *Streptococcus mutans* presented similar biomass quantification for both the test substance and the 2% Chlorhexidine Gluconate gel. This finding is different from that reported by Da Silva et al.(2017) whose test with KR12 did not result in significant changes in *S. mutans* biofilm formation.

CONCLUSION

The elucidation and analysis of the results of the present study demonstrated the antimicrobial activity of the 10% *Aloe vera* gel against all the oral bacteria analyzed. Additionally, the antimicrobial activity of the 10% *Aloe vera* gel against the strains of *Streptococcus parasanguinis* and *Lactobacillus rhamnosus* was higher than that of the 2% Chlorhexidine Gluconate gel. The antibiofilm potential of the 10% *Aloe vera* gel did not significantly change the quantification of bacterial biomass even at different times of attack. However, there were changes in the number of the CFU of the mature biofilm counted 24h after contact with the antimicrobial agents, especially for the strains *Streptococcus salivarius*, *Lactobacillus rhamnosus* and *Lactobacillus acidophilus*, against which the activity was greater in comparison with the 2% Chlorhexidine Gluconate gel. However, such a reduction did not affect the amount of biomass.

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REFERÊNCIAS

Socransky, S. S., &Haffajee, A. D. (2005). Periodontal microbial ecology. Periodontology 2000, 38, 135–187. https://doi.org/10.1111/j.1600-0757.2005.00107.x

Donlan, R. M., &Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical microbiology reviews, 15(2), 167–193. https://doi.org/10.1128/cmr.15.2.167-193.2002

Obst, U., Schwartz, T., & Volkmann, H. (2006). Antibiotic resistant pathogenic bacteria and their resistance genes in bacterial biofilms. The International journal of artificial organs, 29(4), 387–394. https://doi.org/10.1177/039 139 880 602900408

Jones C. G. (1997). Chlorhexidine: is it still the gold standard?. Periodontology 2000, 15, 55–62. https://doi.org/10.1111/j.1600-0757.1997.tb00105.x

Eick, S., Radakovic, S., Pfister, W., Nietzsche, S., &Sculean, A. (2012). Efficacy of taurolidine against periodontopathic species--an in vitro study. Clinical oral investigations, 16(3), 735–744. https://doi.org/10.1007/s00784-011-0567-2

Oosterwaal, P. J., Mikx, F. H., van den Brink, M. E., &Renggli, H. H. (1989). Bactericidal concentrations of chlorhexidine-digluconate, amine fluoride gel and stannous fluoride gel for subgingival bacteria tested in serum at short contact times. Journal of periodontal research, 24(2), 155–160. https://doi.org/10.1111/j.1600-0765.1989.tb00871.x

Arweiler, N. B., Auschill, T. M., &Sculean, A. (2018). Patient self-care of periodontal pocket infections. Periodontology 2000, 76(1), 164–179. https://doi.org/10.1111/prd.12152

Parekh, Jigna, & Chanda, Sumitra. (2007). In vitro antibacterial activity of the crude methanol extract of Woodfordiafruticosa Kurz. flower (Lythraceae). Brazilian Journal of Microbiology, 38(2), 204-207. https://dx.doi.org/10.1590/S1517-83822007000200004

Cragg, G. M., & Newman, D. J. (2013). Natural products: a continuing source of novel drug leads. Biochimica et biophysica acta, 1830(6), 3670–3695. https://doi.org/10.1016/j.bbagen.2013.02.008

Vogler, B. K., & Ernst, E. (1999). Aloe vera: a systematic review of its clinical effectiveness. The British journal of general practice : the journal of the Royal College of General Practitioners, 49(447), 823–828.

Patri, G., &Sahu, A. (2017). Role of Herbal Agents - Tea Tree Oil and Aloe vera as Cavity Disinfectant Adjuncts in Minimally Invasive Dentistry-An In vivo Comparative Study. Journal of clinical and diagnostic research : JCDR, 11(7), DC05–DC09. https://doi.org/10.7860/JCDR/ 2017/27598.10147

Ashouri Moghaddam, A., Radafshar, G., Jahandideh, Y., &Kakaei, N. (2017). Clinical Evaluation of Effects of Local Application of Aloe vera Gel as an Adjunct to Scaling and Root Planning in Patients with Chronic Periodontitis. Journal of dentistry (Shiraz, Iran), 18(3), 165–172.

Moody, J. O., Adebiyi, O. A., & Adeniyi, B. A. (2004). Do Aloe vera and Ageratum conyzoides enhance the anti-microbial activity of traditional medicinal soft soaps (Osedudu)?. Journal of ethnopharmacology, 92(1), 57–60. https://doi.org/10.1016/j.jep.2004.01.018

O'Toole, G. A., & Kolter, R. (1998). Initiation of biofilm formation in Pseudomonas fluorescens WCS365 proceeds via multiple, convergent signalling pathways: a genetic analysis. Molecular microbiology, 28(3), 449–461. https://doi.org/10.1046/j.1365-2958.1998.00797.x

Chandra Shekar, B. R., Nagarajappa, R., Suma, S., & Thakur, R. (2015). Herbal extracts in oral health care - A review of the current scenario and its future needs. Pharmacognosy reviews, 9(18), 87–92. https://doi.org/10.4103/0973-7847.162101

Milovanova-Palmer,J.,Pendry, B.(2018).Is there a role for herbal medicine in the treatment and management of periodontal disease? Journal of Herbal Medicine, 12,33-48.doi:10.1016/j.hermed.2018.02.004

Pugh, N., Ross, S. A., ElSohly, M. A., & Pasco, D. S. (2001). Characterization of Aloeride, a new high-molecular-weight polysaccharide from Aloe vera with potent immunostimulatory activity. Journal of agricultural and food chemistry, 49(2), 1030–1034. https://doi.org/10. 1021/jf001036d

Pandey, R., & Mishra, A. (2010). Antibacterial activities of crude extract of Aloe barbadensis to clinically isolated bacterial pathogens. Applied biochemistry and biotechnology, 160(5), 1356–1361. https://doi.org/10.1007/ s12010-009-8577-0

Habeeb, F., Shakir, E., Bradbury, F., Cameron, P., Taravati, M. R., Drummond, A. J., Gray, A. I., & Ferro, V. A. (2007). Screening methods used to determine the anti-microbial properties of Aloe vera inner gel. Methods (San Diego, Calif.), 42(4), 315–320. https://doi.org/10.1016/j.ymeth.2007.03.004

Lawless J, Allan J. (2000). The clinical composition of *Aloe vera*. London: Thorsons Publishing Ltd.doi: 10.12691/ajphr-4-6-2

Fani, M., & Kohanteb, J. (2012). Inhibitory activity of Aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. Journal of oral science, 54(1), 15–21. https://doi.org/10.2334/josnusd.54.15

da Silva, B. R., de Freitas, V. A., Carneiro, V. A., Arruda, F. V., Lorenzón, E. N., de Aguiar, A. S., Cilli, E. M., Cavada, B. S., & Teixeira, E. H. (2013). Antimicrobial activity of the synthetic peptide Lys-a1 against oral streptococci. Peptides, 42, 78–83. https://doi.org/10.1016/j. peptides.2012.12.001

Evaristo, F. F., Albuquerque, M. R., dos Santos, H. S., Bandeira, P. N., Avila, F., da Silva, B. R., Vasconcelos, A. A., Rabelo, E., Nascimento-Neto, L. G., Arruda, F. V., Vasconcelos, M. A., Carneiro, V. A., Cavada, B. S., & Teixeira, E. H. (2014). Antimicrobial effect of the triterpene 3β,6β,16β-trihydroxylup-20(29)-ene on planktonic cells and biofilms from Gram positive and Gram negative bacteria. BioMed research international, 2014, 729358. https://doi.org/10.1155/2014/729358

Jain, I., Jain, P., Bisht, D., Sharma, A., Srivastava, B., & Gupta, N. (2015). Use of traditional Indian plants in the inhibition of caries-causing bacteria--Streptococcus mutans. Brazilian dental journal, 26(2), 110–115. https://doi.org/10.1590/0103-6440201300102

da Silva, B. R., Conrado, A., Pereira, A. L., Evaristo, F., Arruda, F., Vasconcelos, M. A., Lorenzón, E. N., Cilli, E. M., & Teixeira, E. H. (2017). Antibacterial activity of a novel antimicrobial peptide [W7]KR12-KAEK derived from KR-12 against Streptococcus mutans planktonic cells and biofilms. Biofouling, 33(10), 835–846. https://doi.org/10.1080/08927014.2017.1374378

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