

Comparative study between *Aloe vera* (L.) Burm.f. Extract Mouth Rinse and Chlorhexidine Mouth Rinse in Preventing Biofilm Growth on Titanium Dental Implants

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ABSTRACT

The primary concern regarding peri-implant infections is the growth of biofilm on dental implant abutments. It is believed that using antimicrobial mouthwash may reduce biofilm development and subsequent infections. This study aimed to assess the anti-biofilm properties of *Aloe vera* extract (AVE) against *Streptococcus mutans* biofilm cultivated on titanium dental implant discs. The experiment utilized medical-grade titanium dental implant discs measuring 15 mm in diameter. To determine the phytochemical profile of AVE, its constituents were analyzed using gas chromatography-mass spectrometry (GC-MS), identifying various bioactive compounds, including anthraquinones, flavonoids, and saponins, known for their antimicrobial effects. The study involved sampling *St. mutans* from a healing abutment on a freshly placed dental implant and culturing it on titanium discs in vitro. The titanium specimens were inoculated with bacteria and incubated at 37 °C for 24 hours, after which they were treated with chlorhexidine gluconate (CHX) (n = 5) or AVE (n = 5). Antibacterial activity tests revealed that both AVE and CHX significantly inhibited bacterial growth compared to the negative control. The levels of lactate and turbidity were notably lower in both treatment groups than in the control (p < 0.05). In the antibiofilm testing, there was a statistically significant reduction in turbidity for biofilms in both treatment groups. Scanning electron microscope (SEM) images showed minimal bacterial adherence to the titanium discs in the treatment groups, while confluent and integrated biofilms were observed on the discs in the control groups. These results suggest that *A. vera* extract possesses significant antimicrobial and antibiofilm properties against *Streptococcus mutans* and could be clinically utilized as a natural mouthwash alternative.

Keywords: *Aloe vera* extract, Antibiofilm, Natural mouthwash, *Streptococcus mutans*, Titanium dental implant

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Introduction

One significant risk factor that can compromise the effectiveness of dental implant therapy is peri-implant mucositis. If untreated, this condition can progress to peri-implantitis, leading to implant failure and loss of surrounding bone. Peri-implant mucositis is characterized by inflammation of the mucosa surrounding an endosseous implant, without any loss of supporting peri-implant bone [1].

Plaque biofilm is one of several characteristics that might be regarded as risk indicators for peri-implant mucositis [2]. Bacterial biofilms that accumulate around osseointegrated dental implants cause peri-implant mucositis to emerge from healthy peri-implant mucosa [3]. Compared to gingivitis, the prognosis for peri-implant mucositis may be prolonged. It was discovered that peri-implant mucositis can take more than 21 days to become clinically reversible [4]. Nonetheless, biofilm-induced peri-implant mucositis can be considerably reduced with regular dental hygiene procedures. For instance, compared to the periodontal side, it was discovered that following oral hygiene practices, the gingival index of peri-implant mucositis dramatically decreased [4].

Researchers have explored various approaches to cure or prevent peri-implant mucositis. The aim of these interventions is to clean the contaminated surfaces of implants from peri-implant biofilm [5]. Mechanical debridement has been combined with air powder abrasion using glycine powder or chitosan brushes. However, compared to the control group, no significant clinical advantages were observed in terms of the bleeding index (BI) or bleeding on probing (BoP) [6]. Furthermore, in randomized controlled clinical studies evaluating adjuvant therapies such as photodynamic therapy or diode lasers combined with mechanical debridement, BI and BoP did not show significant improvement compared to the control group [7]. Likewise, there was no discernible clinical improvement with supplementary local antiseptics such as sodium hypochlorite or chlorhexidine (CHX) gel and mouthwash [8].

According to this research, the clinical outcomes for peri-implant health do not show significant improvement when traditional mouthwashes are used as supplementary therapy, despite their well-established antibacterial effectiveness in vitro. It has been suggested that alternative antibacterial treatments may yield better results. For instance, studies have shown that non-thermal atmospheric pressure plasma exhibits significantly greater

antibacterial activity than chlorhexidine (CHX) against *Streptococcus mutans* biofilms on titanium implant surfaces [9]. The increasing microbial resistance to CHX, one of the most commonly used antimicrobial agents in dentistry, may be a contributing factor. Recently, there has been growing interest in the antibacterial and anti-inflammatory properties of natural, plant-based products [10].

The anti-plaque effectiveness of herbal mouthwashes has been comparable to that of CHX [11]. *Aloe vera* (L.) Burm.f. extract (AVE) has emerged as a promising alternative due to its antibacterial, anti-inflammatory, and wound-healing properties. These benefits stem from the active compounds found in aloe vera, a succulent plant in the Liliaceae family, including anthraquinones (such as aloin), saponins, salicylic acid, and polysaccharides like acemannan. Numerous studies have demonstrated the antibacterial effectiveness of AVE against oral pathogens, including *Porphyromonas gingivalis*, *Candida albicans*, and *St. mutans* [12, 13]. *A. vera* is an excellent choice for long-term mouthwash use due to its biocompatibility and minimal adverse effects. The liquid form of *A. vera* extract serves as a safe and effective mouthwash for managing peri-implant microbiota, as it reduces the risk of bacterial resistance and avoids the negative side effects associated with traditional antiseptics, such as tooth discoloration and altered taste perception. Therefore, this study aimed to investigate the antibacterial properties of *A. vera* extract against isolated *St.s mutans*, an early colonizer found in the peri-implant region of the oral cavity.

MATERIALS AND METHODS

The Process of Preparing the Specimens

In this experiment, medical-grade titanium alloy discs (Ti6Al4V), commonly used for dental implants, were employed. Laser cutting was utilized to produce circular discs with a diameter of 15 millimeters and a thickness of 1 millimeter. These discs were then polished with sandpapers ranging from 800 to 1200 grit, using a rotary tool (Grinder-Polisher, Buchler, UK Ltd, Coventry, England). To achieve the final polish, a diamond solution with a concentration of 6.5 microns was applied (Diamond solution, Kemet International Ltd, UK). Finally, the specimens were cleaned with a 5% hydrochloric acid solution and an alkaline solution.

A. vera Extract (AVE) Preparation

Mature *A. vera* plants from the Al-Anbar region in western Iraq were harvested for their fresh leaves (Fig. 1). The leaves were thoroughly washed in distilled water to remove any surface contaminants, then peeled to extract the clear gel while discarding the green rind. The gel was combined into a single mixture and filtered through sterile gauze to eliminate any fibrous remnants. The resulting aqueous extract was then heated to a simmer at 50 °C for approximately 15-20 minutes to enhance its stability while preserving its bioactive compounds. This water-soluble *A. vera* extract is widely regarded as soothing and healing, and it has been traditionally used in folk medicine for skin healing, gastrointestinal issues, and oral infections due to its antibacterial, anti-inflammatory, and antioxidant properties.

A.vera Extract (AVE) Chemical Contents Analysis by using GC-MS

The chemical composition of the *A. vera* Extract (AVE) was analyzed using GC-MS. The Agilent 7000 Triple Quad GC

7890A was the chosen instrument for this research. The GC oven was initially set to 220 degrees Celsius, increasing at a rate of 4 degrees Celsius per minute, and maintained at that temperature for ten minutes. It was then raised to 230 degrees Celsius at a rate of 1 degree Celsius per minute. The mass spectrum was scanned from 25 to 800 atomic mass units, with the ion source temperature set at 230 degrees Celsius. Mass spectra were recorded at a total energy of 70 eV.



Fig. 1 *Aloe vera* properties

The Identification and Isolation of *St. mutans* in the Area Surrounding Dental Implant Abutments

The bacterial swab collection was taken from the dental implant healing abutment of the patient, who was a female and was 31 years old (Fig. 2). Following its arrival at the laboratory, the sample was grown on blood agar and then incubated at 37 degrees Celsius for a period of twenty-four hours. The colony that had been discovered on the blood agar was then identified with the help of the VITEK 2 equipment, which was manufactured by bioMerieux in North Carolina, United States.



Fig. 2 Gingival former (healing abutment) around which the microbial swab was collected.

Disc Diffusion Method for Determining the Effectiveness of Antibacterial Agents

The antibacterial activity of AVE was assessed using the disc diffusion method, a reliable technique for evaluating microbial sensitivity to antimicrobial agents. In this experiment, six-millimeter-diameter filter sheets were soaked in AVE (n=5) and CHX (n=5) for four hours. After conditioning, the filter paper

was transferred to Mueller Hinton agar to support the growth of freshly sub-cultured *St. mutans*. The agar plates were examined after twenty-four hours of incubation to observe whether the area surrounding the filter sheets inhibited bacterial growth.

Preparation of a Bacterial Suspensions

All of the microbiological samples that were separated from the dental implant abutment were placed on blood agar and cultivated for twenty-four hours at 37 degrees Celsius in order to obtain bacterial growth. Utilising a swab of the material, the microbial colony that was present in the blood agar was subsequently cultured in brain heart infusion (BHI). It was activated by incubating the BHI sample for twenty-four hours at 37 degrees Celsius. The sample was placed in anaerobic jars.

The Design of Experiments

St. mutans was cultivated on titanium dental implant discs for the primary experiment of this study, which aimed to investigate the antibacterial and antimicrobial properties of AVE and CHX. Each experimental group included a treatment group (AVE), a positive control (0.3% CHX), and a negative control. To initiate the experiment, *St. mutans* was introduced into 1.5 BHI and inoculated onto titanium discs placed in sterile glass tubes. The discs were then cultured in an anaerobic jar at 37 degrees Celsius overnight. After twenty-four hours, the BHI was removed, and the specimens were exposed for sixty seconds to four milliliters of AVE (n=5) and 0.3% CHX (n=5). Following the exposure, the specimens were washed three times with sterile water to eliminate any residual test solutions. They were then cultured for another twenty-four hours with fresh BHI. Finally, the turbidity of the biofilm and the BHI were measured. Additionally, a duplicate set of the control, treatment, and positive control was prepared to examine bacterial morphology using a scanning electron microscope (SEM) after twenty-four hours.

The Utilisation of Optical Density for the Evaluation of Bacterial Growth

Turbidity testing is a common method that is used to determine the amount of bacterial growth in a medium. When the turbidity of the water increases, it is a sign that the bacteria are proliferating significantly. Using a 96-well plate that had a flat bottom and a lid, 100 microlitres of the nutrient broth from the blank, negative control, treatment, and positive control groups were put to the plate. As a subsequent step, 100 microlitres of new BHI was introduced into every well. After that, the absorbance values of the 96-well plate were measured at 630 nm using a plate reader (BioTek ELX800) in order to determine the turbidity of the sample.

Investigation on the Formation of Biofilm on Titanium Implant Discs

The antibiofilm assay conducted in this study followed the methodology described in reference (25). After one day, the media was removed, and the biofilm was stained with 1% crystal violet for ten minutes. The intensity of the stain was then assessed to determine the strength of the biofilm. After staining, the specimens were rinsed with distilled water to eliminate any excess dye and allowed to dry at room temperature. Subsequently, three milliliters of ethanol were used to detach the biofilm from each specimen. The ethanol solution containing the biofilm was then extracted and placed into a 96-well plate. Finally, a BioTek ELX800 plate reader was used to measure the optical density at a wavelength of 630 nm.

SEM Analysis of *St. mutans*' Confluence and Morphology on Titanium Discs

The bacterial cells' morphology was visualised using SEM following the experiment. In situ analysis of the bacterial cells on the titanium discs was conducted. The media were disposed of after the experiment, and phosphate buffer was used to wash the specimens. Subsequently, the discs' bacterial biofilm was submerged in ethanol solutions (30, 50, 70, and 95%) for 20 minutes each, followed by one hour in 100% ethanol. After being allowed to dry overnight, the specimens were sputter-coated with chromium (26). SEM was then used to analyse the bacterial cells on the discs for confluence and morphology.

Analytical Statistics

Using Stat Graphics version 16, the data are analysed and presented as mean $\pm$ S.E.M. Data were first subjected to a one-way ANOVA, followed by Tukey's test, in order to identify any significant differences between the groups. P values less than 0.05 were regarded as statistically significant, and all statistical analyses employed a 95% confidence level.

RESULTS

AVE's Chemical Analysis

GC-MS was used to analyse the aqueous (AVE) chemical content. Nine chemical structures were discovered during the 12.3–18.3 minute study (Fig. 3). Table 1 lists the names of chemical structures along with their start, end, and current time.

Table 1 Chemical names and structures found in AVE by GC-MS.

Peak Name	Start	End	Real Time
3-Cyclohexene-1-methanol, 2-hydroxy-alpha, alpha,4-trimethyl-	15.19	15.34	15.25
5-Bornanediol	15.5	15.64	15.52
Pterine-6-carboxylic acid	18.23	18.42	18.32
Boronia butenal	16.29	16.45	16.36
Acetate	16.91	17.05	16.98
Oleamide	17.28	17.44	17.36
Undecanol	17.29	17.45	17.36
Dieproxyhexadecan	14.82	15.08	14.92
Aspidospermidin	17.95	18.04	17.96

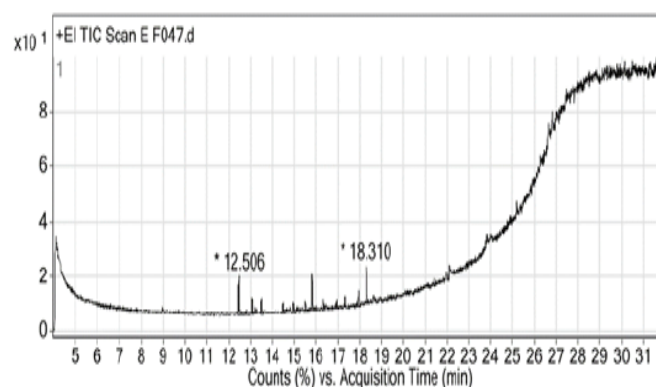


Fig. 3 The observed peaks in AVE using MC-MS as a percentage vs acquisition time.

Examination of the peri-implant Sample Microbiologically

After a 24-hour incubation period in blood agar, the peri-implant abutment swab was subjected to microbial identification analysis using VITEK 2. According to the results, the colony was 96% likely to be *St. mutans*.

AVE and CHX's Antibacterial Activities Utilising the Disc Diffusion Method

The Disc Diffusion Method was used to investigate the test materials' antibacterial activity. The CHX-induced growth inhibition zone for *S. mutans* was discovered to be approximately 4 mm. However, the growth restriction zone surrounding AVE-conditioned discs was just 2 mm. The difference between them was statistically significant ($P \leq 0.05$) (Fig. 4).

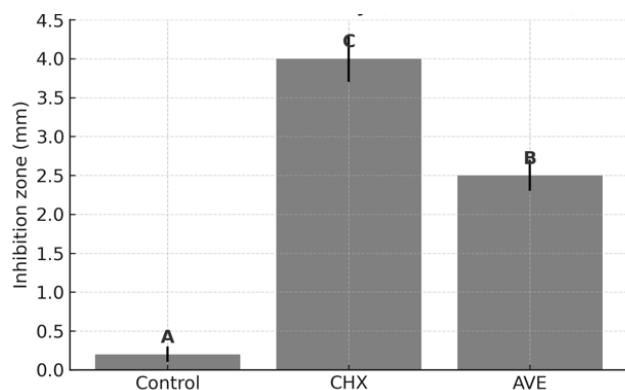


Fig. 4 The inhibitory zone of *St. mutans* surrounding CHX and AVE-conditioned discs on agar plates

Evaluation of AVE and CHX's Antibacterial and Antibiofilm Properties Against *St. mutans* Cultured on Titanium Discs

For one minute, grown *St. mutans* on titanium discs were exposed to AVE and CHX. The data indicated that *St. mutans* survived longer in AVE compared to CHX; however, bacterial growth was significantly lower in AVE than in the control.

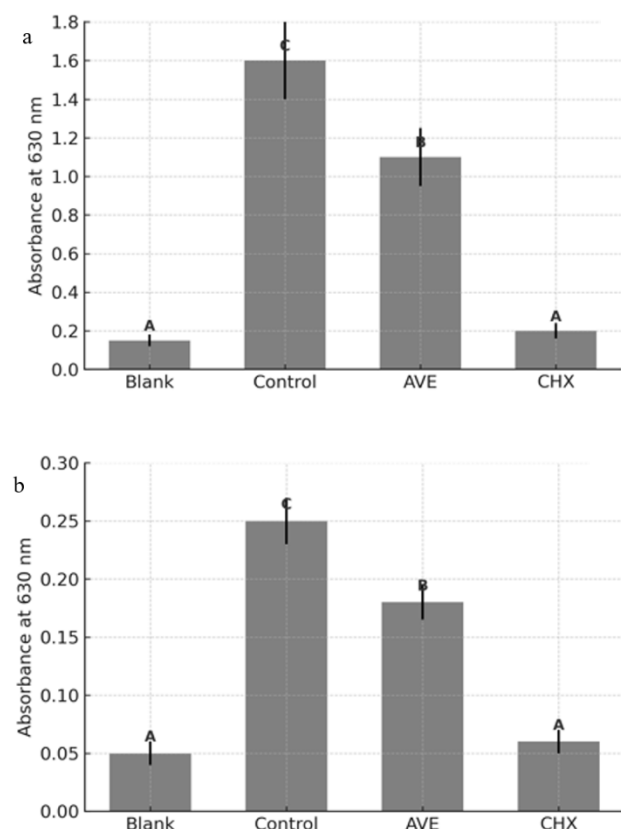


Fig. 5 Antibacterial and antibiofilm activity evaluation. Turbidity values of isolated *St. mutans* inside BHI (a) and *St. mutans* inside the biofilm (b).

Figure 5A shows that the turbidity in AVE was considerably higher than in CHX ($P \leq 0.05$), while the turbidity of suspended bacteria in BHI was significantly greater in the control group than in the other groups. The turbidity values for control, AVE, and CHX were 0.27, 0.19, and 0.05, respectively, highlighting the antibiofilm activity. There was a significant difference between AVE and CHX, with the control group showing a markedly higher turbidity than the others ($P \leq 0.05$) (Fig. 5b).

In the lactate production experiment, the control group showed a significantly higher level of lactate generation compared to the other groups. However, the levels of lactate produced by the AVE and CHX in suspension were not significantly different, with measurements of 7, 4, and 0.5 μM for the three groups, respectively, as illustrated in Figure 6 A. In contrast, the amount of lactate produced by *St. mutans* in the biofilm was much higher in the control group, measuring 3, 1, and 0.2 μM for AVE and CHX, respectively, as shown in Figure 6 B. Additionally, the morphology and confluence of *S. mutans* on titanium discs were examined using scanning electron microscopy (SEM).

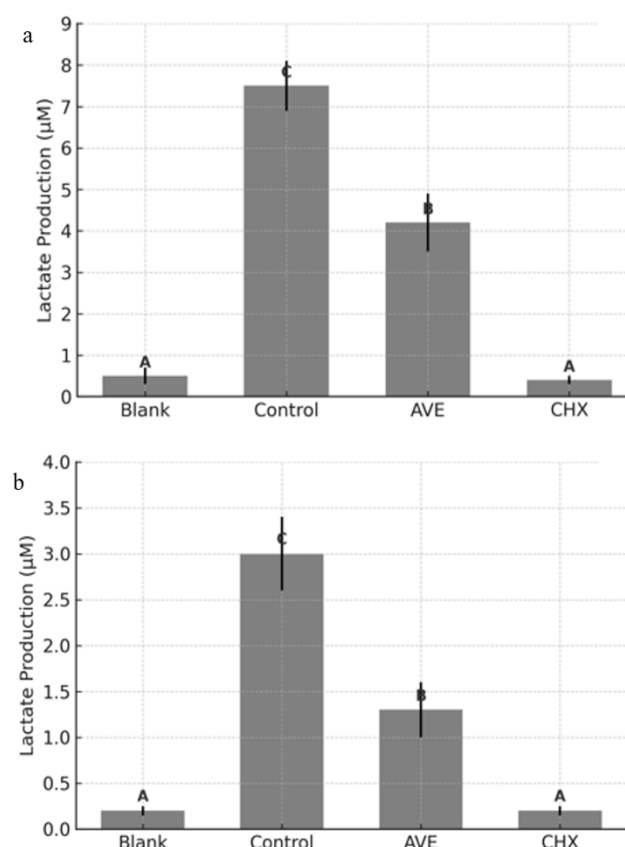


Fig. 6 Lactate synthesis by *St. mutans* in BHI (a) and inside the biofilm (b).

Figure 7 A shows that the control group's bacterial cells covered the substrate in a confluent manner. In contrast, the CHX group did not show any cells at all (Fig. 7 b), and following AVE treatment, only few cells clung to the substrate (Fig. 7 c).

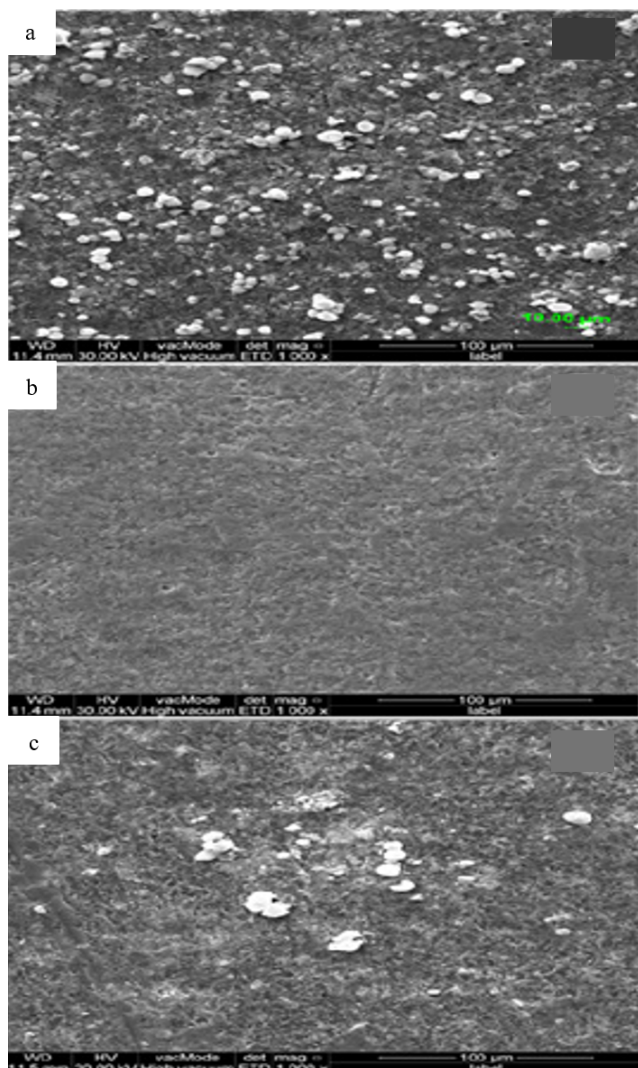


Fig. 7 Images from SEM of titanium disc-grown *St. mutans*. Control (a), CHX (b), and AVE (c). While there are very few cells on AVE and no cells on CHX, observe the confluence and cell proliferation on the control.

DISCUSSION

Extensive research has focused on oral hygiene products that eliminate bacteria around dental implants. This research highlights the need for effective, safe, and affordable alternative mouthwashes, particularly due to rising bacterial resistance to antibiotics, the undesirable side effects of some existing antimicrobial mouthwashes in dentistry, and financial constraints in developing countries [14]. The goal is to create an antimicrobial mouthwash that kills harmful bacteria without harming the good bacteria in the mouth [15]. This study investigated the effectiveness of two commonly used mouthwashes in dentistry, AVE and CHX, against *St. mutans*. The primary focus was on AVE as a naturally occurring, commercially available mouthwash that could help prevent illness caused by microbial colonization around dental implants. The research employed AVE in its water-based form, and its chemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The analysis identified nine distinct chemical components in the sample.

The chemical content of *A. vera* extract (AVE) may vary based on the sample's chemical form. This research identified pterine-6-carboxylic acid as one of the compounds present. Pterine-6-carboxylic acid, which possesses antibacterial properties, is found in many medicinal plant extracts, including *A. vera*.

Therefore, it is reasonable to suggest that the presence of pterine-6-carboxylic acid contributes to the antibacterial characteristics of AVE, given its well-documented antibacterial activity [16]. Not only that, but other investigations [17] have also discovered oleamide in AVE, and it is well-known that both of these compounds possess antibacterial characteristics. The oral cavity's peri-implant abutment was the source of the microbes utilised in this investigation. *St. mutans*, an early coloniser around dental implants, was identified in the peri-implant swab study.

The initial colony on the implant is established by *St. mutans*, which sets the stage for subsequent colonizers like *P. gingivalis* and *P. intermedia*. To prevent peri-implantitis and mucositis around implants, it is crucial to inhibit *St. mutans*. In comparative studies, chlorhexidine (CHX) achieved a 4 mm growth inhibition around CHX-conditioned discs, while AVE produced a smaller inhibition zone of only 2 mm. Furthermore, research on AVE's antibacterial and antibiofilm effects against *St. mutans* on titanium discs showed that AVE significantly increased bacterial damage compared to the control, whereas CHX notably reduced bacterial damage (Figure 5-7). Overall, the results indicated a substantial reduction in the number of germs in saliva after using herbal formulations compared to chemical controls [18]. This conclusion aligns with another study that examined the antibacterial activity of natural products against *St. mutans*. Additionally, a separate study found that *A. vera* gel effectively reduced the concentration of *St. mutans* in the saliva of orthodontic patients. Researchers discovered that patients who used *A. vera* as a mouth rinse experienced a significant decrease in the levels of *St. mutans* in their saliva [19]. More than twice as much growth inhibition of *St. mutans* was observed in the *A. vera* extract group as in the control group, according to another study [20].

The use of medicinal plants in the treatment of different ailments has been the subject of numerous reports [21, 22]. There is substantial evidence that *A. vera* inhibits the growth of *St. mutans*. However, the effectiveness of *A. vera* extract (AVE) against peri-implant microbiota—the bacterial biofilm that forms on implant surfaces—has not been tested. To replicate an in vivo (clinical) environment, this study cultivated bacteria on titanium dental implant discs, distinguishing it from similar research in the literature. The presence of *St. mutans* trapped in a biofilm on these titanium discs may explain why AVE exhibited less antibacterial action than chlorhexidine (CHX). Bacterial colonies embedded in a robust biofilm on titanium surfaces are inherently more protected from antibacterial agents compared to those that develop on agar plates [23, 24]. Compared to the negative control, AVE significantly inhibited the growth of *St. mutans*, indicating its potential as a mouthwash to prevent peri-implant mucositis, despite its antibacterial activity being lower than that of CHX. As an all-natural alternative to synthetic mouthwashes, AVE offers a variety of pharmacological and biological benefits. Using AVE as a mouthwash has several advantages over conventional chemical mouthwashes, including fewer side effects, additional health benefits for patients, and lower costs. However, further research, particularly clinical trials, is needed to determine the effectiveness of AVE mouthwash in reducing peri-implant mucositis.

CONCLUSION

This paper demonstrates that *A. vera* extract (AVE) possesses significant antibacterial and antibiofilm properties against

St.mutans, a key early colonizer in peri-implant biofilm formation. Although AVE is less effective than chlorhexidine (CHX), it significantly prevents the growth and biofilm accumulation on titanium implant surfaces when compared to the control. This antimicrobial activity may be attributed to biologically active compounds such as pterine-6-carboxylic acid and oleamide. Given its natural origin, biocompatibility, and lower risk of side effects, AVE presents a promising alternative to conventional mouthwashes for preventing peri-implant mucositis. Further clinical trials are recommended to validate these findings and explore its potential for daily dental care.

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