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Research Article
Purification, Characterization and Antibiofilm Activity of Ascorbic Acid Extracted from Banana Peels *Musa paradisiaca* L.

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Abstract

Background and Objective: The nutritional benefits of bananas make them a popular food. Studies from the past have shown that banana plants and their fruits can be utilized to cure medical conditions in people. Dental caries is mostly caused by *Streptococcus mutans*. This investigation aimed to quantify the amount of ascorbic acid present in banana peels following solvent extraction. **Materials and Methods:** The extraction of ascorbic acid from banana peels was carried out with methanol treatment and purified with silica gel column chromatography. The characterization of ascorbic acid was performed by FTIR, HPLC and TLC analysis detection of antibiofilm activity of ascorbic acid against dental caries-causing isolates. Statistical analysis on $p \leq 0.05$ was used to compare separate groups with each other. **Results:** The ascorbic acid was purified from methanolic extract and the ascorbic acid-specific O-H groups, C=O groups and C=C groups in FTIR were determined and high-performance liquid chromatography with a retention duration of 6.4 min was used to confirm ascorbic acid. Also, in TLC the purified ascorbic acid had one spot at the same level as standard ascorbic acid with Rf value of 0.80. After being isolated from plaque dental decays, *S. mutans* and one isolate, *S. sobrinus*, displayed varying degrees of biofilm formation. With inhibition percentages ranging from 39-60%, ascorbic acid demonstrated antibiofilm efficacy against various pathogens. **Conclusion:** Ascorbic acid can be utilized to develop dental formulae that inhibit biofilm formation, which can be used for therapeutic purposes or in routine oral hygiene practices.

Key words: *Streptococcus* spp., *Musa paradisiaca* L., biofilm, ascorbic acid, banana peels

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Dental caries development is associated with the creation of biofilms and affects a vast global population. It is thought that *Streptococcus mutans* bacteria are the main etiologic factor causing this dangerous illness[1]. For the oral mucosa and tooth enamel to create multi-dimensional, complex structures, *S. mutans* is a necessary component[2]. Certain cariogenic characteristics are acquired by it, having the ability to adhere to solid surfaces, populate the oral cavity and withstand the acidic environment found there[3].

The three-dimensional complex structure known as oral biofilm is made up of many microorganisms that live in the oral cavity. The biofilm can grow and potentially cause dental cavities if treatment is not received for a long time[4]. Exopolysaccharide matrix biofilm-associated microorganisms are more dangerous because they are more resilient to the immune system of the host and other antimicrobial therapies[5].

Ascorbic acid, also known as vitamin C (AA, C_6H_8O) is one of the most important food ingredients that is soluble in water and is essential to numerous bodily functions[6]. Three methods exist for producing vitamin C: Chemical synthesis, bacterial oxidative fermentation or natural source extraction[7]. Worldwide, bananas are tropical fruits that are grown in many different nations and belong to the Musaceae family[8]. The banana plant has medical uses for all of its parts, including the blossom, pulp, stem and leaves[6]. Studies have revealed that banana peels also have therapeutic qualities. Banana peels are a waste product of bananas[7,8]. The manufacture of several hormones, flavonoids and other developing processes, as well as the growth of plants, division of cells, expansion of cell walls and expression of genes, are all influenced by ascorbic acid[8]. It is a significant antioxidant that is elevated in plants as a defense strategy against environmental stressors like drought. Ascorbic acid is also an essential component of the network of plant antioxidants that detoxify H_2O_2 to combat oxygen radicals[9].

Among the world's most significant crops for human nutrition and mineral content is the banana. Numerous studies have revealed that the peel and pulp of bananas have high-value nutritional components that are used in the pharmaceutical industry, such as carotene, phenolic compounds like gallocatechin and vitamins A, B, C and E[10]. Thus, this study looks at the amount of ascorbic acid in banana peels, purifies and characterizes it and then assesses how effective it is at preventing the formation of biofilms using biofilm formers taken from dental plaque.

MATERIALS AND METHODS

Study area: The study was conducted in the Teaching Laboratories in Medical City and Ibn-El Balady Hospital from February to April, 2024.

Preparation of row powder: To create powder dust, the 200 g of banana peels were cut into tiny pieces, dried and crushed.

Preparation of banana peel extract: With a Soxhlet extractor (Quick fit, UK), the extraction ascorbic acid procedure was carried out. In the flask beneath the extraction chamber, there were roughly 5 g of dried banana peels and 200 mL of methanol. After heating the flask to 55°C, the extraction procedure took 7 hrs to complete. To obtain the concentrated extract, the extracts were collected after 7 hrs and evaporated using a rotary evaporator (Quick fit, UK). The quantity of vitamin C was calculated. Ethanol, hexane and toluene were the three different solvents used to perform the identical processes[8].

Ascorbic acid content: Utilizing a modified colorimetric technique, the ascorbic acid level was found. Addition of 500 µL of the Folin-Ciocalteu reagent (10%, v/v) to the 900 µL of trichloroacetic acid (10% w/v) to which a 100 µL sample had been added. An absorbance measurement at 760 nm was made 10 min later. A standard curve of ascorbic acid was used to find the concentration[6].

Purification of ascorbic acid: To separate the ascorbic acid from the methanolic extract, column chromatography in silica gel was used. Hexane was used to produce the silica slurry, which was poured from the top of the column about two-thirds of the way down while the solvent was simultaneously drained off to help with correct column packing. Hydrochloric acid was utilized for the elution process. For every fraction, at 760 nm the absorbency was read.

Analysis of ascorbic acid

High-Performance Liquid Chromatography (HPLC): High performance liquid chromatography (Knauer, European) was used to find out if ascorbic acid was present in addition to the standard ascorbic acid as a reference. An analytical column in reverse phase (Nucleosil-C18 25×0.4 mm) was used. In isocratic elution, a combination of 95% aqueous NaH_2PO_4 (0.05 M) and 5% methanol is used as the mobile

phase. The mixture was adjusted to pH 4 with 85% phosphoric acid. About 242 nm was used as the wavelength for detection. About 20 µL as an injected volume and 0.7 mL/min as the flow rate of mobile phase[11]. The retention time was utilized to identify ascorbic acid.

FTIR spectra: A FTIR spectrophotometer (Knauer, Germany) was applied to record the FTIR spectra of both the extracted and standard ascorbic acid, ranging from 4000-400 cm^{-1}.

Thin layer chromatography: There were spots of extract and standard on the chromatographic plates. The chromatographic plates were dried and then developed using a 7:3 methanol-to-water ratio as the mobile phase. Monitor the development until the front of the solvent is approximately 1 cm from the plate's top. Once removed, the plate dries out. Fluorescence quenching under UV light at a wavelength of 254 nm was used to identify the ascorbic acids[12].

Isolation of *Streptococcus* spp.: A homogenous suspension of twelve dental decay plaque samples was obtained by vortexing the samples in phosphate-buffered saline for a minute. Mitis salivarius bacitracin (MSB) agar was used to plate the samples, which were mixed with the identical buffer. The mitis salivarius agar (MSB agar) is supplemented with 15% sucrose, 1.5% agar, 0.01% potassium tellurite solution and 0.2 units/mL of bacitracin[13]. Anaerobic incubation of the plates was conducted for 48 hrs at 37°C. The colonies were identified after the incubation period based on their morphology and biochemical tests were used to confirm the diagnosis[14].

Detection biofilm formation: The prepared culture of each isolate with brain heart infusion broth supplemented with 2% sucrose was adjusted to 0.5 McFarland and then divided into 200 µL wells each well on 96 flat bottom microtiter plates. Following a 48 hrs incubation period at 37°C, after the bacterial culture was removed from the microtiter plate by inverting it, the plates were washed three or four times using sterile saline. The cleaned wells were then filled with 200 µL of methanol and the plates were left to stand at room temperature for 20 min. Following that, the plates were left to dry and the fixative methanol was decanted. Following three rounds of distilled water washing and an additional 15 min to allow the plates to stand at room temperature while inverted to stain each well, 200 µL of crystal violet was applied. After each well received 200 µL of 33% glacial acetic acid, the adhering cells and their biofilms were reconstituted. The OD at 600 nm was then measured using a microplate reader (Huma Reader, Germany). According to Zayed *et al.*[15] the biofilm production was calculated. Biofilm strengths are <0.120 for weak, 0.120-0.320 for moderate and ~0.320 for strong.

Assessment of purified ascorbic acid as antibiofilm agent: To assess the effectiveness of purified ascorbic acid against biofilm producers using the microtiter plate method. A particular bacterial culture was mixed with 100 µL of ascorbic acid in 5 mM phosphate buffer and the mixture was incubated for 48 hrs at 37°C. Ascorbic acid was replaced with 100 µL of 5 mM phosphate buffer in the negative control. Following the incubation period, the previously described biofilm activity was carried out once more. For every pathogenic bacterium, the proportion of biofilm development was calculated using the formula[15]:

$$\text{Biofilm inhibition } (\%) = 1 - \frac{\text{Average OD 600 of treated isolate}}{\text{Average OD 600 of untreated isolate}} \times 100$$

Statistical analysis: The gathered data were subjected to a One-way Analysis of Variance (ANOVA) test to compare separate groups with each other. The findings were presented as Mean±Standard Error (SE) with $p>0.05$ regarded as statistically non-significant and $p\leq0.05$ as significantly different.

RESULTS AND DISCUSSION

Screening of ascorbic acid of banana peel extracts: After extracting the ascorbic acid using several solvents, its concentration was ascertained. As can be seen in Fig. 1, the ascorbic acid concentration rose as the solvent's polarity increased. When compared to other solvents like ethanol, toluene and hexane, methanol exhibits the highest concentration of ascorbic acid, reaching 5.17 mg/L. Because hexane was less polar than methanol, which was the most polar solvent, it indicated a lower ascorbic acid level.

The polarity of the solvent is crucial to the extraction process because it boosts the yields of polar compounds[15]. Since ascorbic acid is a polar molecule, the solvent with a higher polarity is required to produce the maximum ascorbic acid yields[16]. This phenomenon explains the increase in ascorbic acid concentration that resulted from the extraction procedure as solvent polarity increased. Ascorbic acid extracted from the method increased with a drying temperature up to 60°C but dropped with a drying temperature up to 70°C[8].

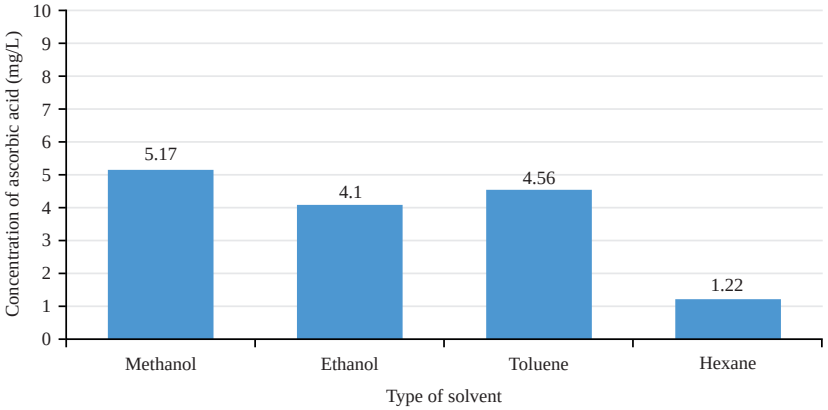

Fig. 1: Extraction of ascorbic acid from banana peels by various solvents

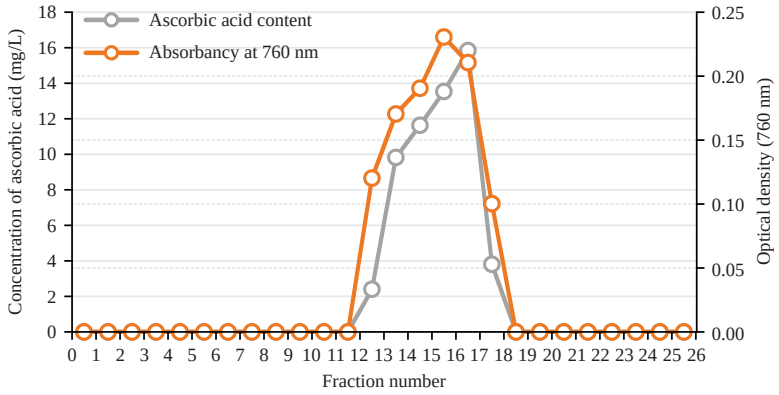

Fig. 2: Purification of ascorbic acid by silica gel chromatography

Purification of ascorbic acid: The concentration of ascorbic acid was evaluated by testing the fractions that were recovered from the methanolic extract of banana peels prepared using silica gel column chromatography so that, as shown in Fig. 2, the ascorbic acid level reached 12.7 mg/L.

Analysis of ascorbic acid

High-Performance Liquid Chromatography (HPLC): Based on HPLC analysis, the standard ascorbic acid had a relative peak occurred at 6.2 min retention time (Fig. 3a), while purified ascorbic acid displayed a maximum peak at 6.4 min retention time (Fig. 3b).

Utilizing High-Performance Liquid Chromatography (HPLC) to characterize ascorbic acid from dog rose fruit, the ascorbic acid was detected after a 6.15 min retention period, as reported by Said *et al.*[10].

FT-IR spectra: According to the results in Fig. 4(a-b), the FTIR spectra of the extracted and standard ascorbic acid showed that three absorption bands that show the presence of O-H groups developed at 3520, 3423 and 3320 cm^{-1}. In addition to 1668 cm^{-1} peak associated with the C=C group, there was also an additional peak at a location of 1750 cm^{-1} related to the group of C=O (Fig. 4b) when compared to the peaks of standard ascorbic acid (Fig. 4a).

The various hydroxyl groups are represented by the peaks in the ascorbic acid IR spectra that fall between 3215 and 3520 cm^{-1}[17].

The ascorbic acid spectrum's distinctive absorption peak at 1750 cm^{-1} is caused by stretching vibrations of the five-membered lactone ring's C=O[18].

Thin layer chromatography: Figure 5 shows the findings of thin layer chromatography for the standard, extracted and purified ascorbic acid. It shows that the methanolic extracts of banana peels had two spots, while the purified ascorbic acid had one spot at the same level as standard ascorbic acid with an Rf value of 0.80.

The mixture of n-butanol was chosen for TLC analysis of apple, kiwi and lemon juice extract with mobile phase

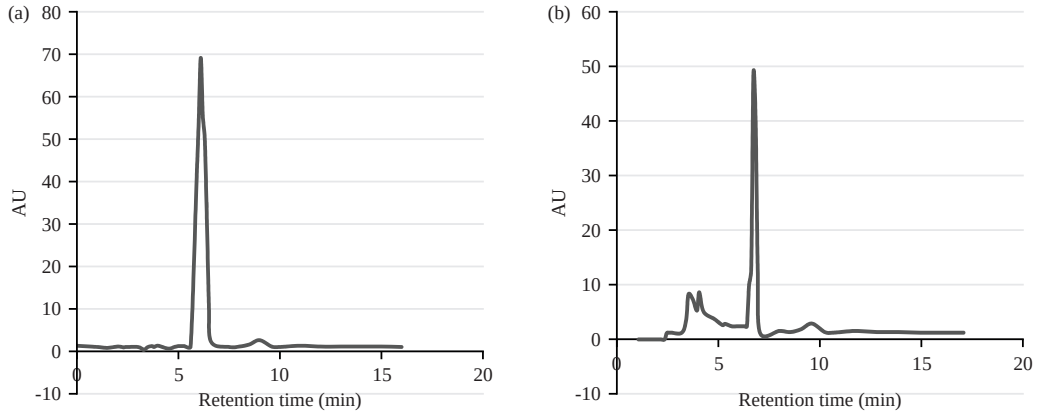

Fig. 3(a-b): HPLC analysis for ascorbic acid from banana peels, (a) Standard ascorbic acid and (b) Purified ascorbic acid

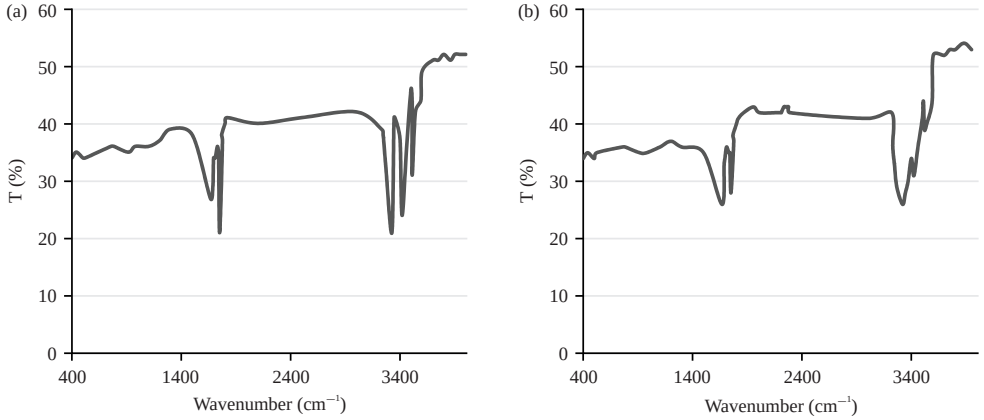

Fig. 4(a-b): FTIR spectra for ascorbic acid from banana peels, (a) Standard ascorbic acid and (b) Purified ascorbic acid

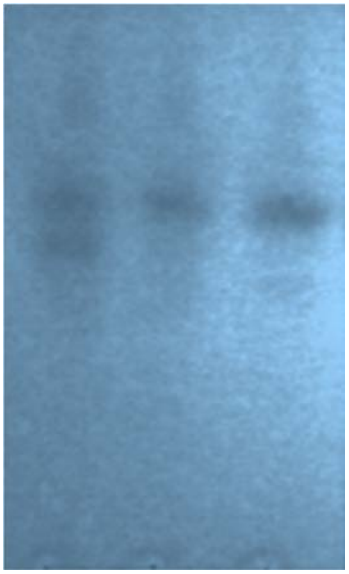

Fig. 5: Thin layer chromatography for ascorbic acid from banana peels

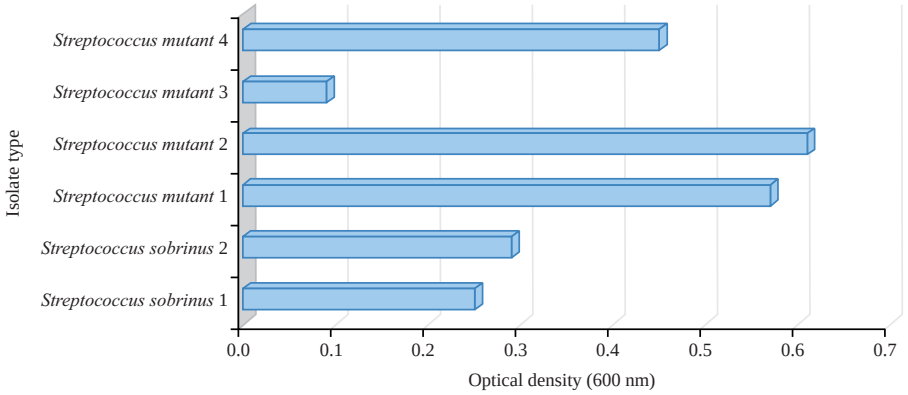

Fig. 6: Screening biofilm formation by *Streptococcus* spp.

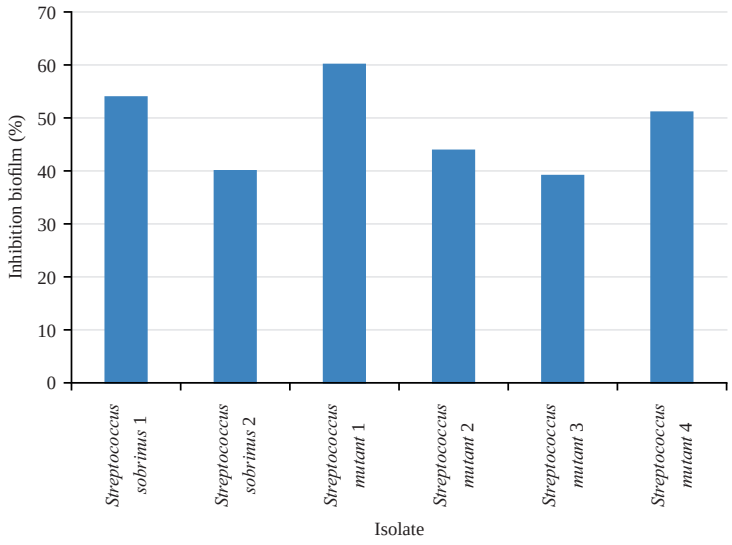

Fig. 7: Detection of biofilm inhibition rates by ascorbic acid for *Streptococcus* spp.

employed in this determination, which included chloroform: acetic acid:ammonia:water with Rf values of 0.83, 0.85 and 0.81 was obtained using water with a ratio of 7:4:5:1:1[14].

Detection of biofilm formation by *Streptococcus* spp.: Five isolates of *Streptococcus* spp., including four isolates of *S. mutans* and one strain of *S. sobrinus*, were recovered after culturing twelve plaque samples from dental decays. Microtiter plates were used to evaluate these isolates for their ability to form biofilm. The results were shown in Fig. 6, where it was shown that three *S. mutans* isolates were strong biofilm producers and one isolate was weak, while both *S. sobrinus* isolates were moderate producers.

Streptococcus mutans, facultative anaerobic bacteria, is the main factor causing dental caries, which is one of the most common diseases affecting the mouth. It is crucial for the development of dental caries, which can worsen and result in infective endocarditis. It does this by facilitating the formation of biofilm and promoting microbial attachment to tooth enamel and each other. Thus, one of the hardest issues facing oral dentists today is preventing this illness[19].

The capacity of *S. mutans* to produce glucan, a component of the polysaccharide matrix that enhances attachment and adhesion efficiency, from sucrose is primarily responsible for its adherence and attachment to tooth enamel[20]. By creating hydrogen bonds with a salivary pellicle and other bacteria, the glucan improves *S. mutans'* adherence, boosting the host defense and biofilm tolerance to several chemotherapeutic drugs[20].

Assessment of purified ascorbic acid as an antibiofilm agent: The antibiofilm properties of pure ascorbic acid derived from banana peels were assessed using a microtiter plate assay, employing specific test isolates of *Streptococcus* spp.,

obtained from dental decay specimens. The outcome demonstrated ascorbic acid's ability to inhibit *Streptococcus* species through antibiofilm activity. Inhibition percentages varied from 39-60%, as shown in Fig. 7. Inhibiting the formation of oral biofilm introduced throughout the inoculation period was possible using ascorbic acid.

When ascorbate and chlorhexidine were present, the cellular debris on a black backdrop was primarily the DNA of dead microorganisms with dye accumulations, which confirmed the information from earlier MTT tests, these dye accumulations vary morphologically from bacterial biofilm. The 16 hrs after the inoculation, ascorbate was unable to separate the biofilm, indicating that the antibacterial action is what kills the cells to prevent the formation of biofilms[21].

Extracting ascorbic acid from banana peels (*Musa paradisiaca* L.) presents a sustainable solution for antibiofilm treatment, with potential in healthcare, food preservation and industry. However, challenges include extraction efficiency, stability and bioavailability. Improving extraction methods, creating stable formulations and exploring synergistic effects with other agents could boost its efficacy against biofilms. Limitations of ascorbic acid from banana peels include low extraction yield, instability to light and heat, limited bioavailability, variability in composition and reduced effectiveness against mature biofilms and multiple microbial species. However, it is recommended to improve ascorbic acid's antibiofilm efficacy from banana peels (*Musa paradisiaca* L.), optimize extraction methods, enhance stability through formulations, explore combination therapies, develop targeted delivery systems and expand research to test its effectiveness on diverse microorganisms and applications.

CONCLUSION

The production of glucan by *S. mutans* and *S. sobrinus* facilitates the formation of biofilm and promotes microbial attachment to tooth enamel and each other. This leads to dental caries, also known as infective endocarditis. Ascorbic acid, which is derived from banana peels, has antibiofilm properties against *Streptococcus* species, which increases its importance for preventing oral infections. It is possible to create dental formulas that prevent the production of biofilms by using ascorbic acid.

SIGNIFICANCE STATEMENT

The ascorbic acid may be used in the creation of a new dental formula that prevents biofilm formation by many causes of dental decay plaques. So that this study was performed to determine the amount of ascorbic acid in banana peels, purify and characterize it and then assess how effective it is at preventing the formation of biofilms using biofilm producers collected from dental plaque. According to the obtained results *S. mutans* and *S. sobrinus* were isolated from dental plaques and revealed different levels of biofilm formation and an application of ascorbic acid displayed varying degrees of biofilm inhibition. This finding refers to using ascorbic acid as an antibiofilm agent against various pathogens that infect the mouth.

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