

## Dental Biofilm Composition in Situ and Enamel Demineralization Affected by *Psidium Cattleianum* Leaf Extract

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### Abstract

Dental caries develops when sugar-fermenting dental biofilms are actively active, but the most effective methods for controlling it only target mineral loss. Decreased salivary stream rates (hyposalivation) essentially worsen caries movement by diminishing sugar and corrosive leeway close to tooth surfaces. Keeping the health of the dental biofilm symbiosis (health) under hyposalivation necessitates knowing how acid inhibition affects specific dietary regimens.

*Psidium cattleianum* leaf extract has not previously been evaluated under conditions that were comparable to the oral environment.

**Keywords:** Agents that kill bacteria; Dental decay; Extract of plants

### Introduction

Biofilms are microbial communities embedded in a polymeric matrix attached to the surface of the tooth [1]. The disease appears as a result of a breakdown of biofilm homeostasis, which from the interaction of specific bacteria with constituents of a diet in a According to, a mouthwash containing propolis can reduce the formation of supragingival plaque and insoluble polysaccharides. Smullen et al.4 show that a wide range of extracts, including tea, red and green grape extracts, cocoa, and tea, can kill *Streptococcus mutans*. Until now, most investigations of normal items against oral microorganisms endeavor to confirm their antibacterial properties and systems of activity in vitro [2].

When the restorative properties of a therapeutic plant are observationally known, the World Wellbeing Association starts the pharmacological assessment of phytotherapeutic drugs from such sources. In spite of current interest in their utilization in medication, under 15% of therapeutic plants from tropical regions that have the potential for remedial use have been studied.

*Psidium cattleianum* is ordinarily known as strawberry guava [3]. It is native to tropical America and is a member of the Myrtaceae family. *Psidium* species (*Psidium* spp.) plants are used to treat a number of diseases around the world, and the antibacterial properties of these plants have been tested against bacteria that cause diarrhea or opportunistic infections. Previous research has shown that the leaf extracts of plants in this family, like *P. guajava*, can also reduce gingivitis and halitosis. Previous research has also shown that *P. cattleianum* leaf extract is effective against *S. mutans* biofilms and explained how it works. Be that as it may, this concentrate has not been assessed in that frame of mind to the oral climate. Using in situ caries models, dental products can be evaluated to help predict clinical outcomes and evaluate numerous aspects of dental biofilm and hard tissues.

Using an in situ dental caries model, the purpose of this study was to determine how *P. cattleianum* leaf extract affected enamel demineralization and the biochemical and biological composition of oral biofilm.

Dental caries happens because of the disintegration of polish, brought about by acids delivered by the dental biofilm [4]. Effective fluoride conveyed by brushing with toothpaste is acknowledged as viable in the avoidance of caries<sup>1</sup>, fundamentally in light of the fact

that it diminishes the pace of lacquer demineralization and improves the pace of finish remineralization. Various in vitro examinations have shown that the pace of caries movement and abatement is straightforwardly impacted by the fluoride content of the fluid period of the biofilm overlying.

In practice, a biofilm 2 covering a tooth surface makes it susceptible to caries. Subsequently biofilm liquid is the key dissolvable in which minerals are traded at the tooth surface [5]. It appears that its anti-caries effect 7 is directly related to the level of fluoride in biofilm, specifically in biofilm fluid.

Fluoride in the biofilm liquid exists in harmony with fluoride in the spit 8, which thusly exists in balance with fluoride bound to the oral delicate tissues 9, which are accepted to be the key fluoride supply in the wake of brushing. Both biofilm fluid fluoride levels and saliva fluoride levels are important for caries prevention because of their interdependence.

### Materials and Methods

**Extract preparation:** The reaping of *P. cattleianum* leaves was approved by the Brazilian Climate and Regular Assets Establishment. The University of Campinas's Collection of Aromatic and Medicinal Plants received a voucher herbarium specimen.

For the purpose of extract preparation, only healthy leaves (those without signs of necrosis and with typical color and shape) were chosen. After being washed in tap and deionized water for one week, the leaves were dried at 37 °C [6]. After that, the leaves were ground in a blender into a fine powder. After decocting the solution in 100 g/600 mL of deionized water for 5 minutes at 100 °C and 55 °C for 1 hour, the solution was filtered and sterilized using 0.22 m mixed cellulose ester

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membranes. The concentrate was put away in dim containers at  $-20^{\circ}\text{C}$  until additional utilization.

**Veneer block arrangement and investigation:** Lacquer blocks estimating  $4\text{ mm} \times 4\text{ mm}$  were acquired from cow-like incisor teeth recently put away in 2% formaldehyde arrangement (pH 7.0) for 1 month<sup>16</sup> and had their surfaces sequentially cleaned [7]. Using a Shimadzu HMV-2000 microhardness tester, the surface microhardness (SMH) and cross-sectional enamel microhardness (kg mm<sup>2</sup>) were measured. According to Vieira et al.<sup>17</sup>, for baseline SMH (SMH1), five 100  $\mu\text{m}$ -diameter indentations were made in the center of the enamel block using a 25-g load for ten seconds. Following the experimental phase, SMH was measured once more (SMH2). Five spaces divided 100  $\mu\text{m}$  separated and from the standard were made. The rate change of SMH (% $\Delta\text{SMH}$ ) was determined as follows: % SMH is equal to  $100 (\text{SMH2} - \text{SMH1})/\text{SMH1}$ .

The blocks were longitudinally sectioned through the center for cross-sectional microhardness tests. The cut face of one of the halves was left exposed and gradually polished after it was embedded in acrylic resin.

The mean qualities at every one of the 3 estimating focuses at each separation from the surface were then arrived at the midpoint [8]. The Knoop hardness number; the integrated hardness The integrated loss of subsurface hardness (KHN) is the enamel subsurface demineralization area after the in situ experiment.<sup>18</sup> KHN is calculated by subtracting the integrated hardness of sound enamel from the KHN (m) of the lesion into sound enamel.

**Exploratory plan:** This review was recently endorsed by the human moral council from Araçatuba Dental School (convention #2005-02188). Ten healthy volunteers between the ages of 23 and 34 were chosen. The crossover in situ experiment consisted of three phases, each lasting 14 days and corresponding to the treatment solution: The volunteers used non-fluoridated toothpaste one week prior to and throughout the experiment.<sup>19</sup> 20 deionized water (negative control), concentrate, or Unique Listerine Antiseptic™. Due to the physical differences in the solutions used (i.e., color, smell, and taste), the in situ phase of the study was not blind. However, during the crossover study analysis, it was possible to make the study blind. After the experimental phase, the enamel blocks were coded, and the researcher who performed the analysis was unaware of which group an enamel block belonged to.

One millimeter was left between the enamel and the plastic mesh so that biofilm could grow, and the volunteers wore palatal appliances with four enamel bovine blocks covered in plastic mesh to allow for biofilm accumulation and protect it from disturbances.

Polish blocks were randomized by the mean of the SMH from all blocks and its certainty spans. The confidence intervals were calculated using Microsoft Excel 2003. For this experiment, the significance level was set at  $p = 0.05$  [9]. The treatment groups' enamel blocks were distributed so that each group's mean SMH was within the 95% confidence interval for the total block mean. As a result, the median value for each group was set at 381.1 KHN or 378.1 KHN.

After removing the palatal appliance from their mouths, the volunteers applied one drop of 20 percent sucrose to each enamel block eight times per day. The workers held up 5 min prior to returning the machine to their oral cavity.<sup>22</sup> During the fourth and eighth sucrose applications, deionized water, concentrate, or Unique Listerine Antiseptic™ was dribbled 1 min after sucrose application two times per day and the apparatus was supplanted in the oral hole 4 min later.

Between each phase, a washout period of seven days was allowed.<sup>21,23</sup> The volunteers received written and verbal instructions to wear the appliance all the time and take it off for meals or oral hygiene. During the experiment, they were not permitted to use fluoride or antimicrobial products.

**Appraisal of biofilm acidogenicity:** pH was estimated using biofilms and a palladium microelectrode during an overnight fast to ensure that no bacterial carbohydrates were stored and to assess the treatments' residual effects [10]. A salt scaffold between the reference terminal and the worker's finger was made with 3 mol L<sup>-1</sup> KCl.<sup>25</sup>

Two circumstances were assessed during pH estimations. In the first place, on the twelfth day, just sucrose was applied ("sucrose alone" estimation). The pH was estimated 5 min after sucrose application with the apparatuses put in the oral pit. This was done to determine whether pH variations were caused by a structural change in the biofilm caused by the treatment solutions and to assess the potential residual effect of the treatment solutions. Second, on the thirteenth day, sucrose was applied and the treatment arrangements were dribbled ("sucrose + treatment" estimation) after 1 min. The mouth appliances were replaced four minutes later, and the pH was measured. This was done to see how quickly the treatment solutions worked.

Two pH readings were taken: prior to dripping any solutions (baseline measurement) (pH1), as well as after dripping sucrose alone (on the 12th day) or sucrose plus treatment solution (on the 13th day) (pH2). The following was the pH variation calculation:  $\text{pH equals pH1, pH2}$ .

**Examination of dental biofilm structure:** Toward the finish of the trial, the plastic cross section was taken out and biofilms were gathered and gauged. The biofilm was diluted in phosphate-buffered saline to approximately 5 mg (PBS: 8.0 g NaCl, 0.2 g KCl, 1.0 g Na<sub>2</sub>HPO<sub>4</sub>, and 0.2 g KH<sub>2</sub>PO<sub>4</sub> per liter, acclimated to pH 7.4; 1 mL mg<sup>-1</sup> biofilm) and sonicated on ice in a ultrasonic cell disruptor (XL; To analyze total anaerobic microorganisms (TM), total streptococci (TS), and mutans streptococci (MS), respectively, the suspensions were diluted in PBS and plated in duplicate in Mitis salivarius agar, brain heart infusion agar, or Mitis salivarius sucrose bacitracin agar.<sup>27</sup> The presence of TS and MS was confirmed by Gram staining. After 72 hours of incubation at 37 °C in an anaerobic jar, colonies-forming units (CFU) were counted [11]. The outcomes are shown in terms of log CFU mg<sup>-1</sup> wet weight.

Phosphorus pentoxide was used to dry the remaining biofilm.<sup>22</sup> EPS were extracted from the biofilm by adding 1.0 mol L<sup>-1</sup> NaOH (10 L mg<sup>-1</sup> dry weight). The examples were vortexed for 1 min; After three hours of agitation at room temperature, they were centrifuged for one minute at 11,000 g.<sup>28</sup> The supernatants were precipitated overnight with 75 percent cooled ethanol, centrifuged, and resuspended in 1.0 mol L<sup>-1</sup> NaOH.<sup>29</sup> The phenol-sulphuric acid procedure was used for the carbohydrate analysis.<sup>30</sup> The results are expressed in g mg<sup>-1</sup> dry weight.

## Statistical analysis

We hypothesize that treatment with *P. cattleianum* leaf extract reduces enamel demineralization and microbial viability in biofilms. For each experimental phase, the means of the results from each participant's four enamel blocks were calculated. GraphPad Prism Version 3.02 was used for the statistical analysis. Equal variances (Bartlett's test) and a normal distribution (Kolmogorov-Smirnov test) were observed in the microorganism counts (TM, TS, and MS) and percentages of SMH, KHN, EPS, and pH data; Tukey's multiple

comparison test was followed by an analysis of variance (ANOVA) on them. A two-tailed paired t-test was used to analyze pH data collected between the 12th and 13th days. As far as possible was set at 5%. The analyzed parameters' correlations were established.

## Results

Microorganism counts were affected by *P. cattleianum* leaf extract. There were fundamentally less suitable microorganisms in the biofilms after treatment with the concentrate than after treatment with water or dynamic benchmark groups ( $p < 0.05$ ) for TM, TS and MS. After treatment with water or the active control, there were no statistically significant differences in MS viability ( $p > 0.05$ ). For TM and TS, there were statistically significant differences between the water and active control groups. TM was more helpless than TS or MS, as shown by the distinction in log decrease noticed.

Contrasted with water,  $\Delta$ pH was essentially lower with the concentrate and dynamic control in both sucrose alone and sucrose + treatment arrangements; However, the pH drop did not differ significantly between these two conditions ( $p > 0.05$ ). When compared to the active or negative controls, the use of the extract significantly reduced the amount of EPS ( $p < 0.05$ ). After treatment with water or the active control, EPS did not differ significantly ( $p > 0.05$ ).

The present study looked at how *P. cattleianum* leaf extract affected anticaries [12]. The phenolic compounds found in *P. cattleianum* all possess antibacterial activity: 3 are flavonoids (kaempferol, quercetin, and cyanidin) and 1 is a tannin (ellagic corrosive). There are no significant amounts of fluoride, calcium, or phosphate in the examined extract (data are not shown because the levels were below the detection limit). The purpose of employing fluoride-free dentifrice was not to overestimate the extract's efficacy but rather to evaluate its potential effect on its own. This is consistent with the literature and allowed for better scientific control of the study. Further examinations are expected to assess the antibacterial impact of the concentrate contrasted with fluoride items.

Albeit this is whenever an antibacterial substance first has been tried utilizing an in situ caries model, this model has been widely examined to assess the microbiological and biochemical piece of biofilms<sup>34</sup>, microbial destructiveness factors the cariogenicity of food varieties, sugars, and different substances and the defensive impacts of milk items and biting gum. According to these studies, this model can be used to evaluate antibacterial substances. In addition, this in situ model was chosen due to its promotion of a high cariogenic challenge, which results in enamel demineralization; this was extremely valuable for testing the concentrate after the past in vitro, featuring the propensity of the concentrate to diminish corrosive creation.

Our group's previous research demonstrates that *P. cattleianum* leaf extract can prevent the expression of proteins involved in general metabolism, particularly those involved in *S. mutans* biofilms' carbohydrate metabolism [13-16]. A caries rat model was used to confirm the extract's anticariogenic effect in recent research. The current review addresses a transitional stage between in vitro and in vivo examinations. In situ, tests permit the investigation of research center discoveries in a circumstance that imitates the improvement of normal caries while utilizing delicate location techniques.

## Conclusion

The findings confirm that hyposalivation has a significant impact

on biofilm dysbiosis by reducing the number of sugar exposures per day that drive dysbiosis and that the inhibition of acid production slows the rate of biofilm conversion from a symbiotic state to dysbiosis, which may reduce biofilm cariogenicity over time. From a clinical perspective, limiting fermentable sugar intake is essential to control biofilm dysbiosis under hyposalivation, and the findings suggest that even severe inhibitions of biofilm metabolism may not be sufficient to combat the harmful effects of hyposalivation.

## Acknowledgement

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## Conflict of Interest

None

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