Antimicrobial Activity and Antiadherent Effect of Peruvian *Psidium guajava* (Guava) Leaves on a Cariogenic Biofilm Model

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ABSTRACT

Aim: Phytomedicine has been commonly practiced as a form of traditional medicine in various cultures for the treatment of oral diseases. Recently, it has gained importance as an alternative to conventional treatment. Several extracts of plants and fruits have been recently evaluated for their potential activity against microorganisms involved in the development of dental caries. The purpose of this study was to evaluate the antimicrobial activity and antiadherent effect of the crude organic extract (COE) and three partitions (aqueous, butanolic, and chloroformic) of *Psidium guajava* (guava) leaves on a cariogenic biofilm model.

Materials and methods: Guava leaves were obtained from the mountains of northern Peru, where they grow wild and free of pesticides. The antimicrobial activity of the COEs and partitions against *Streptococcus mutans* and *Streptococcus gordonii* was determined by measuring the inhibition halos, while the effect on biofilm adhesion was determined by measuring the optical density using spectrophotometry.

Results: An antibacterial effect of the COE and chloroformic partition against *S. gordonii* ($p < 0.05$) was found, as was a significant effect on biofilm adherence, with a minimum inhibitory concentration (MIC) of 0.78 mg/mL, which was maintained throughout the 7 days of evaluation.

Conclusion: We conclude that the COEs and their chloroformic partition have antimicrobial and antibiotic effects against this strain of *S. gordonii*, making them of particular interest for evaluation as a promising alternative for the prevention of dental caries.

Clinical significance: By knowing the antimicrobial effect of *Psidium guajava*, this substance can be effectively used in products aimed to prevent dental caries and periodontal disease.

Keywords: Cariogenic biofilm, Dental caries, *Psidium guajava*, *Streptococcus gordonii*, *Streptococcus mutans*.

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INTRODUCTION

Bacteria can live in a planktonic state or form biofilms; when the latter occurs, there is an increase in virulence as well as resistance to antibacterial substances, producing different defense mechanisms against the host immune response.[1,2] In the search for new molecules with therapeutic effects, different natural products have been tested for their antioxidant, anti-inflammatory, anesthetic, and antimicrobial effects, with the latter probably being the most widely studied.[3–6] The oral cavity is home to many saprophytic bacteria that contribute to the physiological activities of the host, so it is not always appropriate to use antibacterial agents that affect the balance of this group of microorganisms, and treatments with specific or selective mechanisms should be used instead.[7–10]

The use of naturally derived products for research studies is increasing, aiming to find new molecules with some type of therapeutic effect. Both Peruvian and foreign plants have been investigated. Guava (*Psidium guajava*) is native to the Americas. It grows in tropical areas, and in Peru, it is grown in areas such as San Martín, Loreto, Huánuco, Junín, Lima, and Cusco. It is traditionally used as a medicinal plant for its antidiarrheal, antiseptic, astringent, and digestive properties.[9] Guava leaves are considered stimulants and antispasmodics,[9] and the antibacterial components responsible for their activity are quercetin, avicularin (quercetin-3-α-L-arabinofuranoside), and guaijaverin.[11–13]

The use of common plants has guided pharmaceutical research aimed at combating pathogenic microorganisms. *P. guajava* L.

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is a highly versatile plant that has been used as a food and as a therapeutic agent. The roots, bark, leaves, fruits, flowers, and

seeds are used for different purposes, especially in infusions and decoctions for oral and topical use. *P. guajava* is used in the treatment of symptoms related to organ malfunction and diseases caused by the action of pathogens and/or opportunistic microorganisms. Many pharmacological studies have been carried out scientifically to evaluate the therapeutic potential of this plant.[14,15]

Commonly called *guayaba* or *guayabo*, the guava is a tropical shrub that grows up to 7 m in height. It has a short trunk, flaky bark, and open canopy. Its leaves are leathery with prominent veins, and its flowers are white. Its berrylike fruit has firm pulp and many seeds, with a flavor that is sweet to very acidic, and can be pear shaped, round, or ovoid.[6,16–19]

The chemical constitution of this plant includes tannins, phenols, flavonoids, saponins, carbohydrates, alkaloids, sterols, terpenoids, and phenolic compounds. Compounds of known antimicrobial activity, such as 1,2-benzenedicarboxylic acid, dibut, α-bisabolol, 1,2-benzenedicarboxylic acid, buty, hexadeca-2,6,10,14-tetraen, caryophyllene, germacrene, quercetin, quercetin-3-O-α-L-arabinofuranoside, quercetin-3-O-β-D-arabinopyranoside, morin-3-O-lyososide, morin-3-O-arabin S, quercetin and quercetin-3-O-arabinoside, 11-hydroxy-35-tricont-pentatriacontane, hexaeicosan-16-ol, tricoslan-17-en-5-ol, and nonacosane-23-en-3-ol, are found in the leaves of *P. guajava*.[20–26]

Due to the medicinal importance of *P. guajava*, several studies related to its ethnobiological history, as well as scientific research related to the confirmation of its general pharmacological effects, have been performed.[12,27–30] There are still few research studies on the use of this plant against oral pathogens and even fewer against oral biofilms, although antibacterial activity against cariogenic microorganisms has been evaluated in one study, which found that the effect on *Lactobacillus acidophilus* is similar to the effect of chlorhexidine.[31] Shetty et al.[32] evaluated the inhibitory effect of guava extracts on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Ethanolic and aqueous extracts of guava were prepared, and the inhibitory effects of these extracts on the two periodontal pathogens were tested. *P. gingivalis* was more susceptible to the ethanolic extract than to the aqueous extract. When both bacteria were compared, *A. actinomycetemcomitans* was more susceptible to guava extracts than *P. gingivalis*. Thus, guava extract can be a potential therapeutic agent for periodontitis since it shows significant activity against *P. gingivalis* and *A. actinomycetemcomitans*.

Limsong et al.[33] evaluated the antimicrobial activity of extracts derived from different natural products, including *P. guajava* L., which showed antimicrobial activity against *Streptococcus mutans* with inhibition halos greater than 10 mm. Although it did not present a statistically significant difference from 0.12% chlorhexidine, this fact represents an important finding justifying the study of other species of natural origin in the search for new anticariogenic agents. The present study can lay a foundation for the generation of therapeutic products that can be applied to suppress the formation of biofilms instead of modifying the bacterial ecosystem by killing the saprophytic microorganisms that form the ecosystem.

MATERIALS AND METHODS

Design of the Study
This study had an *in vitro*, prospective, cross-sectional, and analytical experimental design.

Sample Collection
Ten kilograms of leaves of *P. guajava* was collected during the month of November 2018 in rural areas of the city of Oxapampa, Peru, using latex gloves and taking into account the principles of biosafety. Pruners were used to cut the branches, which were put inside a plastic bag. Then, we selected the best leaves that were intact and clean. Next, all the leaves were placed carefully inside cardboard boxes lined with Kraft paper (Fig. 1). Last, the leaves were packed to be transported to the Chemistry Laboratory of the National University of Engineering in Lima, Peru. Another sample of the plant that included the flower was prepared and transported to Cayetano Heredia University in Lima for subsequent taxonomic identification.

The leaves of the guava plant were identified and authenticated by specialized personnel: Dr Camilo Díaz Santibañez, Professor of Botany and Taxonomy, Lima, Perú. A voucher specimen (Specimen No. PP 2018) was deposited in the Department of Pharmacognosy, Research and Development Laboratory, Peruvian Cayetano Heredia University, Lima, Perú.

Preparation of the Crude Extract and Partitions
Each leaf was washed and then dried in an oven at 37°C. After 24 hours, the samples were left to air dry for an additional 24 hours.

The samples of dried leaves were placed in four 1-L flasks, and 600 mL of analysis-grade ethanol (EtOH) was added. The leaves were left to macerate for 24 hours. Then, the samples were gravity-filtered, and the filtrate was collected in a refractory funnel and placed under a fume hood until the ethanol evaporated. Finally, concentrations of 25 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL were prepared[3,27] (Fig. 2).

To partition the crude extract, 100 mL of methanol was added to solubilize the sample with stirring until the sample was dissolved. Then, 100 mL of chloroform was added, and the sample was allowed to stand for 2 hours until solubilization in this medium. Next, 150 mL of Milli-Q water was added to one column, and the solubilized sample was added. It was allowed to stand for 24 hours, after which two well-defined phases were observed: the aqueous phase and the chloroformic phase. Both phases were separated by decantation, and the chloroformic residue was transferred to the fume hood to evaporate all traces of chloroform.

Then, another column was prepared by adding 600 mL of *n*-butanol, and immediately afterwards, the aqueous residue obtained in the previous process was added. After 24 hours of standing, two phases were formed: an aqueous residue and a butanol residue. They were separated by decantation. The butanol residue was evaporated under a fume hood, and the aqueous residue was lyophilized for one week (Fig. 3). All samples were dissolved in dimethyl sulfoxide (DMSO) and Milli-Q water at a ratio of 1:1 and were delivered to the Laboratory of Bacteriology at the Peruvian Cayetano Heredia University for biological evaluation.

Evaluation of the Antibacterial Effect of the Crude Extract
The strains *S. mutans* ATCC 25175 and *Streptococcus gordonii* ATCC 51656 were activated and cultured for inoculum preparation. The two strains were cultured in brain heart infusion (BHI) medium for 24 hours and then brought to the turbidity of the 0.5 McFarland standard. To evaluate the antibacterial effect, a swab was soaked with the previously prepared inoculum, which was seeded on the agar surface four times. After the agar had stood for 5 minutes, filter

Figs 1A to C: (A and B) Branch collection; (C) Collection of leaves
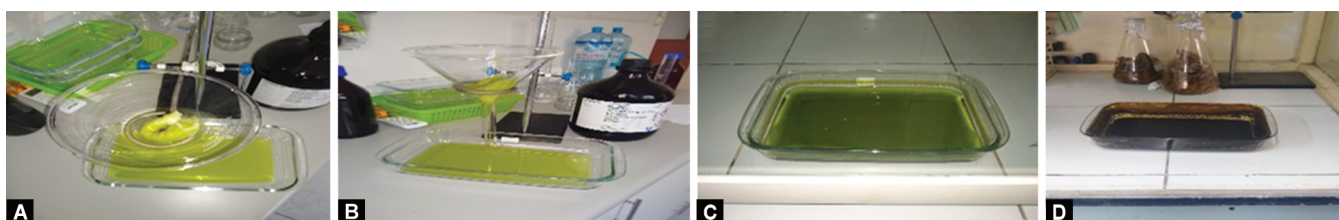
Figs 2A to D: Preparation of the crude extract

Figs 3A to C: Preparation of the partitions: aqueous residue, butanolic residue, and chloroform residue

paper disks (Whatman 3; 6 mm in diameter) were put over the agar and impregnated with 10 µL of each of the guava extracts separately. This procedure was repeated six times. Chlorhexidine (0.12%) was used as a positive control, and the negative control was DMSO diluted 1:1 with Milli-Q water. Then, all the plates were incubated with the indicated concentration of extract, positive control, and negative control at 37°C for 48 hours under microaerophilic (*S. mutans*) or anaerobic (*S. gordonii*) conditions (Fig. 4).

Evaluation of the Antibacterial Effect of the Partitions

To evaluate the partitions of the guava leaves, the following concentrations were tested: aqueous partition, 50 mg/mL (1 mL), butanolic partition, 50 mg/mL (1 mL), and chloroformic partition, 50 mg/mL (1 mL). The bacteria *S. mutans* ATCC 25175 and *S. gordonii* ATCC 51656 cultured in BHI for 24 hours were used, and their turbidity was calibrated to the 0.5 McFarland standard (Fig. 5).

Antibacterial testing was performed by immersing a swab in the previously prepared inoculum. The agar was swabbed four times and left to stand for 5 minutes, and then, 6-mm filter paper disks (Whatman 3) were placed on the agar and impregnated with 10 µL of each of the guava partitions separately. This procedure was repeated six times.

Chlorhexidine (0.12%) was used as a positive control, and the negative control was a solution of DMSO plus Milli-Q water (1:1). Then, all the plates with the partitions were incubated, with positive and negative controls, at 37°C for 48 hours under microaerophilic (*S. mutans*) and anaerobic (*S. gordonii*) conditions. After 48 hours of incubation, the plates were examined, and the inhibition halos were measured with Vernier calipers.

Determination of the Minimum Inhibitory Concentration

After determining the antibacterial effects of the crude extract and partitions of guava, the concentration that showed the highest inhibition halo in *S. gordonii* was used to determine the minimum inhibitory concentration (MIC).

A total of 140 µL of trypticase soy broth (TSB) was placed in 96-well microtitration plates from wells 2B to 11B, and then, 140 µL of the natural guava extract was placed in well 2B. Using a micropipette, 140 µL from well 2B was placed in well 3B and then homogenized, and the same method was repeated until reaching well 11B to complete the 140 µL. Subsequently, 20 µL of the culture of the *S. gordonii* bacteria adjusted to the 0.5 McFarland standard was added from wells 2B to 11B. Then, the plate was incubated
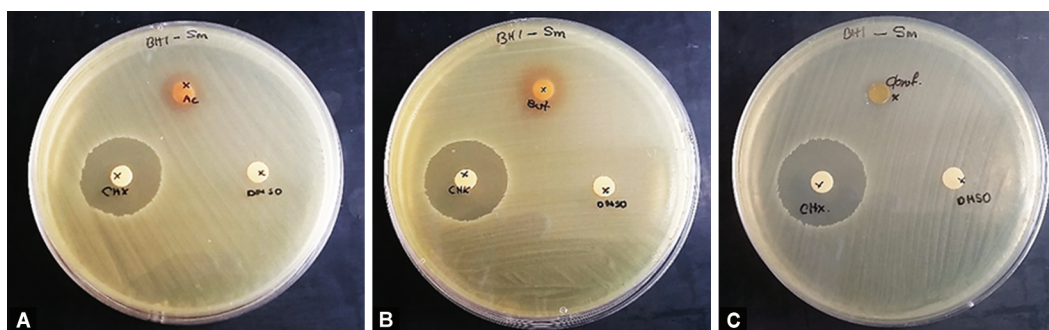
Figs 4A to C: Agar plates with guava partitions at 50 mg/mL and 0.12% chlorhexidine and DMSO controls against *S. mutans*: (A) Aqueous plus controls; (B) Butanolic plus controls; (C) Chloroform plus controls
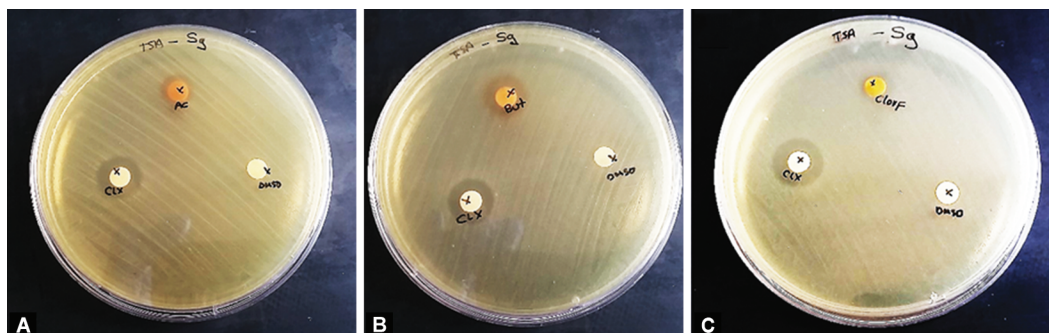
Figs 5A to C: Agar plates with guava partitions at 50 mg/mL and 0.12% chlorhexidine and DMSO controls against 50 mg/mL *S. gordonii*: (A) Aqueous plus controls; (B) Butanolic plus controls; (C) Chloroform plus controls

at 37°C for 48 hours under anaerobic conditions. Chlorhexidine (0.12%) was used as a positive control, and the negative control was DMSO:Milli-Q water (1:1). The procedure was repeated 10 times.

The MIC was determined based on the cell density in the untreated (turbid) well. To verify bacterial vitality, 5 μL from each well was seeded in Trypticase soy agar (*S. gordonii*) such that the concentration at which there was no colony growth was considered the MIC.

Formation of the Biofilm

To form the biofilm, BHI broth was supplemented with 2.5% sucrose (30 g/L in distilled water), artificial saliva (350 mL of a saline solution containing 350 mL of distilled water with 3.15 g of NaCl), a solution of carboxymethyl cellulose (4 g of CMC in 100 mL of distilled H_2O), and 50 mL of glycerin. The final volume was 500 mL. The medium was autoclaved and stored at 4°C.

The biofilm was formed on the surface of the coverslips (10 × 10 mm) placed inside the wells of sterile 24-well microplates. The biofilm was formed with strains of *S. gordonii*, as it was the only bacterium evaluated on which the extract had an effect. It was inoculated in 15 mL of BHI at 37°C under anaerobic conditions for 4.5 hours, until reaching the exponential growth phase, and adjusted to the 0.5 McFarland standard based on the optical density at 550 nm.

The sections of sterile glass slides were placed in the wells of a 24-well microplate, and 300 μL of acclimated sterile artificial saliva was added to each slide and incubated at 4°C for 16 hours. Then, each slide was carefully removed with sterile tweezers and washed gently with 300 μL of phosphate-buffered saline (PBS) (pH 7.0, acclimated) twice and placed in a new sterile well. A total of 280 μL of BHI broth supplemented with 2.5% sucrose was added to each well. Twenty microliters of *S. gordonii* was added to each well, and the turbidity was adjusted to the 0.5 McFarland standard, followed by incubation for 12 hours at 37°C under anaerobic

conditions. After 12 hours, the slides were carefully removed and gently washed with 300 μL of PBS twice. Then, each slide was placed in new sterile wells.

Effect on the Adherence of the Biofilm

To biofilms formed in the previous step, 200 μL of the crude organic extract (COE) of *P. guajava* at a concentration of 0.78 mg/mL was added, which corresponded to the MIC on the surface of the slide, and was left to rest for 1 minute at room temperature. After two washes with 300 μL of PBS, the slides were again placed in new sterile wells. A total of 300 μL of BHI supplemented with 2.5% sucrose was added to the slides, which were then incubated at 37°C for 12 hours. After 12 hours, the same procedure was repeated until 1, 4, or 7 days of growth were completed.

On day 1, 4, or 7 of incubation, the slides were removed and washed with 300 μL of PBS twice and then placed in a new sterile well. Then, 100 μL of trypsin was added to the surface of each slide, and the slides were incubated for 15 minutes at 37°C. The optical density at 595 nm (OD_{595}) of the detached cells was determined in a spectrophotometer, and the results were recorded for the corresponding analyzes.

To measure the antiadherent effect of the crude extracts, the following groups were formed:

- Group I (natural extract): 200 μL of guava COE was applied to the surface of the slide on the first day of biofilm formation and then every 12 hours, with the OD_{595} read on days 1, 4, and 7 of incubation.
- Group II (positive control): 200 μL of 0.12% chlorhexidine was applied to the surface of the slide on the first day of biofilm formation and then every 12 hours, with the OD_{595} read on days 1, 4, and 7 of incubation.

- Group III (negative control): 200 μL of DMSO plus Milli-Q water (1:1) was applied to the surface of the slide on the first day of biofilm formation and then every 12 hours, with the OD_{595} read on days 1, 4, and 7 of incubation.

To measure the antiadherent effect of the partitions of the guava leaves, the following groups were formed:

- Group I (3 partitions): 200 μL of the guava extract (partition) was applied to the surface of the slide on the first day of biofilm formation and then every 24 hours, with the OD_{595} read on days 1, 4, and 7 of incubation.
- Group II (positive control): 200 μL of 0.12% chlorhexidine was applied to the surface of the slide on the first day of biofilm formation and then every 24 hours, with the OD_{595} read on days 1, 4, and 7 of incubation.
- Group III (negative control): 200 μL of DMSO plus Milli-Q water (1:1) was applied to the surface of the slide on the first day of biofilm formation and then every 24 hours, with the OD_{595} read on days 1, 4, and 7 of incubation.

Data Analysis

The results are presented as the means and standard errors of the means. The statistical analysis was performed with one-way or two-way ANOVA followed by Tukey's multiple comparisons test. The level of significance was 0.05.

RESULTS

Interpretation: The COE of guava showed antimicrobial efficacy against only *S. gordonii*, with an average inhibition diameter of 10.4 mm at a concentration of 50 mg/mL ($p = 0.02$) (Table 1).

Interpretation: The aqueous, butanolic, and chloroformic partitions showed no antimicrobial effect against *S. mutans*. The aqueous and butanolic partitions had no antimicrobial effect against *S. gordonii*. The chloroformic partition of guava presented antimicrobial efficacy against only *S. gordonii*, with an average inhibition diameter of 9.12 mm at a concentration of 50 mg/mL ($p = 0.03$) (Table 2).

The MIC of the chloroformic guava residue in *Streptococcus gordonii* ATCC 51656 was 0.78 mg/mL (Table 3).

There was no significant difference in absorbance among days 1, 4, and 7 of growth for the *S. gordonii* biofilms to which the guava COE was applied (Table 4).

Of the three partitions applied every 24 hours to the *S. gordonii* biofilm, the chloroformic residue achieved the best reduction in the biomass of the biofilm on days 1, 4, and 7 of growth. The butanolic residue was also effective in decreasing the biomass, showing a weaker effect than that of the chloroformic residue but a stronger effect than that of the aqueous residue (Table 5).

DISCUSSION

Optimizing people's health is the main objective of health professionals. Currently, dental infections are a common health problem that affects a majority of people. Dental problems are now thought to lead to systemic problems as well. Primary prevention of oral diseases, although considered the best treatment option available, is often hard to achieve. The imprudent use of synthetic medicines has produced resistance against many bacterial strains, and this kind of medicine has been associated with many adverse effects. Therefore, recent research has focused more on herbal preparation, which is effective and had relatively few possible side effects.[34]

P. guajava is a phytotherapeutic plant that is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, and fatty acids.[35] Guava leaves are particularly rich in quercetin, a flavonoid known for its spasmolytic, antioxidant, antimicrobial, and anti-inflammatory effects, and guaijaverin, known for its antibacterial action.[36] Guava also offers other health benefits as an excellent source of antioxidants and a good source of vitamin C, with the ability to eliminate free hydrogen peroxide and superoxide anion and inhibit the formation of hydroxyl radicals.[31,35] Vitamin C, like bioflavonoids, helps accelerate the healing process of tissues. The biologically active components in guava leaves have various systemic and local effects and, since antiquity, have been used in various preparations.[37]

Guava leaf extract has activity against various groups of microorganisms, such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, and *Bacillus cereus*, bacteria that can cause diseases and food spoilage.[29–34] The antibacterial activity of guava leaves has several modes of action. It is suggested that the extract

Table 1: Antibacterial effect of the crude extract of *P. guajava*

| Guava extract/control | Mean diameter of inhibition halos in mm | | |
	S. mutans	S. gordonii	p*
25 mg/mL	0	0	1
30 mg/mL	0	0	1
40 mg/mL	0	0	1
50 mg/mL	0	10.4	0.02
0.12% chlorhexidine	22.3	14.87	0.01
DMSO	0	0	1

* $p < 0.05$ (Tukey's test)

Table 2: Antibacterial effect of *P. guajava* partitions

| P. guajava partition/control | Mean diameters of inhibition halos in mm | | |
	S. mutans	S. gordonii	p*
Aqueous	0	0	1
Butanolic	0	0	1
Chloroform	0	9.12	0.03
0.12% chlorhexidine	23.56	13.72	0.02
DMSO	0	0	0

*$p < 0.05$ (Tukey's test)

Table 3: Minimum inhibitory concentration of the chloroformic residue of *P. guajava* against strains of *S. gordonii*

| Microorganism | A | B | | | | | | | | |
		Concentration (mg/mL)								
S. gordonii	50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	
	n/g	n/g	n/g	n/g	n/g	n/g	n/g	w/g	w/g	

n/g: no growth; w/g: with growth

Table 4: Effect of the crude extract of *P. guajava* on the adherence of *S. gordonii* biofilm

| | OD$_{595}$ | | |
| | Time of evaluation | | |
Extract	1 day	4 days	7 days
Crude organic extract	0.226	0.2031	0.2009
DMSO + H$_2$O	1.513	1.4045	1.3014
Chlorhexidine	0.0565	0.005	0.005
No extract	1.531	1.222	1.065

Table 5: Effect of the *P. guajava* partitions on the adherence of *S. gordonii* biofilm

| | OD$_{595}$ | | |
| | Time of evaluation | | |
Partition	1 day	4 days	7 days
Aqueous residue	1. 088	1.064	0.863
Chloroformic residue	0.271	0.11	0.066
Butanolic residue	0.422	0.533	0.675
DMSO + H$_2$O	1.496	1.114	1. 105
Chlorhexidine	0.047	0.044	0.046
No extract	1.531	1.222	1.065

penetrates the lipid bilayer of the cell membrane, which results in increased permeability, leading to the loss of cytoplasmic content. The tannins present in guava leaf are polyphenolic compounds that bind proline-rich proteins that interfere with protein synthesis, thus exerting antibacterial activity. The flavonoids in guava leaves form complexes with extracellular proteins and are soluble in the bacterial cell wall.[37,38]

In the present study, the antibacterial activity of the COE and guava leaf partitions against cariogenic bacteria and the antiadherent effect of biofilms formed by these microorganisms were evaluated. Initially, antimicrobial assays were performed with the COE at concentrations of 25 mg/mL, 30 mg/mL, 40 mg/mL, and 50 mg/mL against *S. mutans* ATCC 25175 and *S. gordonii* ATCC 51656. The results showed that there was an inhibitory effect against only the first bacterium. On the contrary, when evaluating the effect of the liquid–liquid partitions, an antibacterial effect was observed against only *S. gordonii* with the chloroformic residue at a concentration of 50 mg/mL. Although the antibacterial effect of the control with chlorhexidine was greater, the inhibition halo was much smaller (14.87 mm) than that resulting from the crude extract against *S. mutans* (22.3 mm), which maintained this trend when the partitions were evaluated against *S. gordonii* and *S. mutans*, with inhibition diameters of 23.56 and 13.72 mm, respectively. Preliminary studies[39,40] have identified sterols, flavonoids, alkaloids, saponins, glycosides, and tannins using ethanol (polar) and chloroform (nonpolar) as solvents, while triterpene has only been found when using ethanol. The presence of these phytoconstituents supports the significant bioactivity exhibited by the crude extracts, while the secondary metabolites are associated with many bioactivities that include inhibitory properties against a wide range of pathogens. Among these secondary metabolites, alkaloids have been extensively studied in terms of their antimicrobial activities and their mechanism of action. Similarly, sterols, flavonoids, tannins, saponins, glycosides, and triterpenes have been reported to have significant inhibitory properties against different pathogens.

When the antiadherent activity of the biofilm was evaluated, an effect was found from the first day in the organic crude extract group. Likewise, although all the partitions showed an antiadherent effect, this effect was the greatest for the chloroformic and butanolic residues. Bacteria in most environments form organized communities called biofilms, in which the aggregated cells are embedded in a hydrated extracellular polymeric matrix.[35–38,41,42] Caries is a chronic disease linked to the formation of biofilms. Due to the slow growth, activation of the stress response, induction of the biofilm phenotype, or other aspects of the biofilm architecture, when the cells exist in a biofilm, they can become 10–1,000 times more tolerant to the effects of antimicrobial agents.[42–45] In this context, the COE partitions at a concentration of 0.78 mg/mL had an effect on planktonic cells, but their ability to reduce the *S. gordonii* biofilm biomass was not greater than that of the chlorhexidine

residue. Overall, *P. guajava* inhibited biofilms and biofilm formation, especially the chloroformic residue. The potential of a strain to form a biofilm depends on its capacity for growth and adhesion, that is, the more it grows and adheres to solid surfaces, the better the biofilm formation.[45–50] These data, together with the present results, suggest that the leaves of *P. guajava* reduced the formation of biofilms.

Conclusion

In conclusion, our data demonstrated that the evaluated extracts, both in their crude form and liquid partitions, showed an inhibitory effect on the growth, adherence, and formation of *S. gordonii* biofilms. These data suggest that *P. guajava* could be a possible preventive and therapeutic agent for dental caries.

Availability of Data and Materials

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

Authors' Contributions

Margarita F Requena-Mendizabal and Roger D Calla-Poma carried out the research and wrote the first draft of the manuscript. Ana Valderrama-Negron and Marco Calderon-Miranda assisted in the research and revised the manuscript. Dora Maurtua-Torres revised the manuscript. Pablo A Millones-Gómez guided the research project and revised the manuscript. Pablo A Millones-Gómez and Reyma Bacilio-Amaranto helped with plant collection, cosupervised the research, and revised and submitted the manuscript. All authors read and approved the final manuscript.

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