Natural Resources for Human Health
eISSN: 2583-1194

Original Research

**Received** 08 March 2026
**Revised** 22 April 2026
**Accepted** 10 May 2026

Natural Resources for Human Health 2026, Vol. 6 (3): 456–463

**KEYWORDS**:
Psidium guajava; Oral biofilm; Streptococcus mutans; Candida albicans; Polyphenols; Antibiofilm activity; Oral pathogens; Dental plaque; Phytochemicals; Antimicrobial activity.

# Antibiofilm Activity of Psidium guajava Leaf Polyphenols Against Oral Pathogens

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**ABSTRACT:** Background: A plant-derived antibiofilm fraction may complement conventional plaque-control agents while limiting broad microbial suppression. Objective: To evaluate polyphenol-rich Psidium guajava leaf fraction in comparison with vehicle control and chlorhexidine reference using a in-vitro biofilm experiment. Methods: The draft protocol included 30 experimental units or participants involving single- and dual-species oral biofilms of Streptococcus mutans and Candida albicans. The intervention was administered or tested for 48 hours. The primary outcome was biofilm biomass reduction, with additional assessment of minimum biofilm inhibitory concentration, viable count, extracellular polysaccharide content, surface coverage by confocal microscopy. Standardized laboratory, clinical, or food-analysis procedures were applied, and between-condition effects were evaluated with two-sided tests at $\alpha=0.05$. Results: The Psidium guajava fraction reduced dual-species biofilm biomass in a concentration-dependent manner to 31 ± 5% of control at 100 μg/mL ($p<0.001$).

At 100 μg/mL, extracellular polysaccharide content declined to 37 ± 6% of control and viable S. mutans counts decreased by 2.7 log10 CFU, with substantial thinning of the confocal biofilm architecture. Direction and magnitude of the findings of the dummy were internally consistent across the primary and secondary endpoints, and tolerability or assay quality was acceptable. Conclusions: The example data indicate that the polyphenol-rich fraction of the Psidium guajava leaf might be used to efficiently reduce the biomass of the biofilm by inhibiting glucosyltransferase activity, decreasing microbial adhesion, and interfering with formation of extracellular polymeric matrix. The findings herein are based on a complete manuscript-development example and should be replaced with ethically obtained and independently verified observations before submitting to scientific

publications. Keywords: Psidium guajava; oral biofilm; Streptococcus mutans; polyphenols; antimicrobial.

## 1. INTRODUCTION

Antibiofilm Activity of Psidium guajava Leaf Polyphenols Against Oral Pathogens is an answer to an important question at the intersection between experimental pharmacology and nutritional science, as well as the field of translational health research. Earlier published works directly related to the current study confirmed that polyphenol extract of Psidium guajava leaf has scientific basis for assessment of its role in reducing the amount of biofilm biomass [1]. Yet, these differences in preparation, exposure, comparator choice, and outcome definition remain to be a major obstacle to making confident comparisons across studies.

The following is a second line of evidence for biological plausibility of the present hypothesis. Previous studies on Psidium guajava and similar studies have shown functional or biochemical activity in alignment with the proposed direction of benefit [2]. These observations do not remove the need for a standardized set of materials and criteria for analyzing.

The concepts of reproducibility derived from the wider natural-product and functional-food literature are related to the processing conditions, biological matrix, dose, and chemical characterization of active constituents [3]. The polyphenol rich fraction of Psidium guajava leaf was thus directly used for research purposes and not considered as a crude plant-based product or commercial product.

The proposed mechanism includes glucosyltransferase inhibition, decreased microbial adhesion, and extra cellular polymeric matrix disruption. Related biological effect mechanisms that have been published have typically been complex, multi-pathway interactions of redox, inflammatory, metabolic, microbial, enzymatic, or differentiation pathways, rather than single pathways [4]. Therefore, it is possible to have a more coherent interpretation by measuring a primary functional endpoint along with mechanistic markers.

There is also a need for methods appropriate to the study model in the field of outcome assessment. Laboratory or clinical methodology is emphasized that is established and standardized that samples are handled in a particular way, samples are analyzed in a validated way, appropriate controls are used, and interpretation thresholds are prespecified [5]. These principles guided selection of biofilm biomass reduction as the primary outcome and the accompanying secondary endpoints.

Changes in biofilm biomass reduction are most convincing when they are accompanied by concordant changes in minimum biofilm inhibitory concentration and viable count. Related research has demonstrated the value of combining functional, biochemical, and structural outcomes when evaluating complex botanical, microbial, or food-based interventions [6].

Safety and quality are equally important. Natural origin does not guarantee consistent composition or freedom from adverse effects, contaminants, interactions, or degradation, and published pharmacovigilance and quality-control evidence recommends integrating these considerations from the beginning of product development [7].

Finally, transparent study design is necessary to reduce bias. Guidance and prior methodological research relevant to the present experimental model support randomization, blinding where feasible, replication, complete accounting of exclusions, and reporting of effect estimates rather than reliance on p-values alone [8].

Despite the available evidence, few studies have combined a clearly defined polyphenol-rich Psidium guajava leaf fraction, an appropriate comparator, a prespecified primary endpoint, and supporting mechanistic or feasibility outcomes in single- and dual-species oral biofilms of Streptococcus mutans and Candida albicans. The aim of this study was therefore to determine the effect of polyphenol-rich Psidium guajava leaf fraction on biofilm biomass reduction compared with vehicle control and chlorhexidine reference. Secondary objectives were to evaluate minimum biofilm inhibitory concentration, viable count, extracellular polysaccharide content, and relevant quality or safety indicators.

## 2. MATERIALS AND METHODS

### 2.1. Study design and setting

This manuscript describes a prespecified controlled single- and dual-species biofilm study involving single- and dual-species oral biofilms of Streptococcus mutans and Candida albicans. The planned sample comprised 30 experimental units or participants and was structured to compare polyphenol-rich Psidium guajava leaf fraction with vehicle control and chlorhexidine reference. The time of observation was 48 hours. All the numbers in this paper are dummy/example numbers generated to write the paper, to statistically demonstrate, to form a table, and to create a graph; they are not the numbers of an actual investigation completed. The design is however based on realistic laboratory or clinical workflows such that the draft can be further translated into an approved protocol and substituted with the actual measurements.

### 2.2. Experimental material and test system

The test material was authenticated and standardized before biological testing. Known to have no effect on the assay at the final concentration, polyphenol-rich psidium guajava leaf fraction was prepared in a solvent system. The experimental system used included single and dual species oral biofilm of Streptococcus mutans and Candida albicans. The independent experiments were conducted on different days, and technical replicas of the experiments were within. Appropriate conditions were untreated, vehicle, injury or growth, and reference-comparator conditions.

### 2.3. Concentration selection and exposure

Preliminary range-finding experiment established non-precipitating, assay compatible concentrations and established the upper exposure limit. The primary experiment consisted of a concentration gradient with low, intermediate and high concentrations of the range of concentrations expected to be active. The exposure time was 48 hours. A randomized layout was used to allocate the plates, culture flasks or assay tubes and the positions of the edges were distributed equally across the conditions to reduce systematic plate effects.

### 2.4. Outcome assessment

The main result was a decrease in biofilm biomass in the form of %. Minimum biofilm inhibitory concentration, viable count, extracellular polysaccharide content, surface coverage by confocal microscopy were secondary outcomes. The baseline and follow-up or across concentration and time conditions were to be measured depending on the design. Assays were conducted under proper kit protocols or standard analysis protocols with duplicate measures being made when the coefficient of variation was greater than 10%. Clinical measures were taken following a standardized rest time as well as food-product results were evaluated under standardized conditions of serving.

### 2.5. Quality assurance and bias control

The protocol deviations were addressed in a written laboratory or trial manual that outlined sample labeling procedures, timing, calibration, data entry and protocol violations. In the case where the intervention form allowed it outcome assessors were blinded. A conceptually recheck of ten percent of records against source data was performed, and analytical runs were only accepted when calibration and quality-control samples were within predefined limits. Primary complete-case display did not fill in missing values; sensitivity analyses were performed with an intention-to-treat framework or model-based estimation where necessary.

### 2.6. Sample-size rationale

A sample of 30 units was planned because it is a realistic sample to develop a manuscript. In a real study, the computation can be substituted with one involving a pilot-derived standard deviation and minimal clinically or biologically significant difference in biofilm biomass reduction. The final calculation needs to indicate two-sided $\alpha=0.05$, desired power of at least 80%, allocation ratio, predicted attrition or unusable assays and any correction of repeated measurements, clustering, or multiple primary comparisons.

### 2.7. Statistical analysis

Summarization of continuous data was done as mean Standard deviation when the data were approximately normally distributed and as median with interquartile range when the data were skewed. Categorical results were provided as a number and percentage. Independent t tests, Mann-Whitney tests, chi-square tests, or Fisher exact tests between-group comparisons as required. Primary outcomes were assessed with analysis of covariance, repeated-measures analysis, mixed-effects models, one-way analysis of variance with multiplicity-adjusted post hoc comparisons, or nonparametric equivalents according to design. Effect estimates were accompanied by 95% confidence intervals. Correlations used Pearson or Spearman coefficients. All tests were two-sided, and $p<0.05$ was considered statistically significant. Analyses were conceptually performed in a current statistical package with an auditable syntax file.

## 3. RESULTS

The analysis included the full dummy/example dataset specified for the controlled single- and dual-species biofilm study. Baseline characteristics, assay controls, or starting values were balanced across comparison conditions, and no predefined quality-control run was rejected. The tables present mean ± standard deviation unless another summary is stated. Because the data are illustrative, participant flow, assay exclusions, and p-values were generated to be statistically coherent rather than to represent an actual enrolled sample.

The Psidium guajava fraction reduced dual-species biofilm biomass in a concentration-dependent manner to 31 ± 5% of control at 100 µg/mL ($p<0.001$).

**Table 1.** Antimicrobial and biofilm-inhibitory concentrations

| Organism | MIC (µg/mL) | MBIC50 (µg/mL) | MBIC90 (µg/mL) | MBC (µg/mL) |
|---|---|---|---|---|
| S. mutans | 250 | 46 | 118 | 500 |
| C. albicans | 500 | 82 | 196 | >500 |
| Dual-species biofilm | — | 61 | 154 | — |

Values are dummy/example data presented as mean ± SD unless otherwise stated.

The direction of the primary effect was supported by changes in minimum biofilm inhibitory concentration and viable count. The magnitude of response generally increased with intervention exposure or was greater at follow-up in the intervention arm, while the comparator showed smaller changes. Where repeated measurements were available, the group-by-time or condition effect remained significant after adjustment for baseline values and planned multiple comparisons.

**Table 2.** Biofilm biomass and viable counts

| Condition | Biomass (% control) | S. mutans log10 CFU | C. albicans log10 CFU | p-value |
|---|---|---|---|---|
| Vehicle | 100 ± 0 | 7.8 ± 0.4 | 6.7 ± 0.3 | — |
| 25 µg/mL | 78 ± 7 | 7.2 ± 0.4 | 6.3 ± 0.4 | 0.041 |
| 50 µg/mL | 55 ± 6 | 6.4 ± 0.5 | 5.8 ± 0.4 | 0.004 |
| 100 µg/mL | 31 ± 5 | 5.1 ± 0.5 | 4.9 ± 0.5 | <0.001 |
| Chlorhexidine | 18 ± 4 | 4.3 ± 0.4 | 4.1 ± 0.4 | <0.001 |

Values are dummy/example data presented as mean ± SD unless otherwise stated.

At 100 µg/mL, extracellular polysaccharide content declined to 37 ± 6% of control and viable S. mutans counts decreased by 2.7 log10 CFU, with substantial thinning of the confocal biofilm architecture.

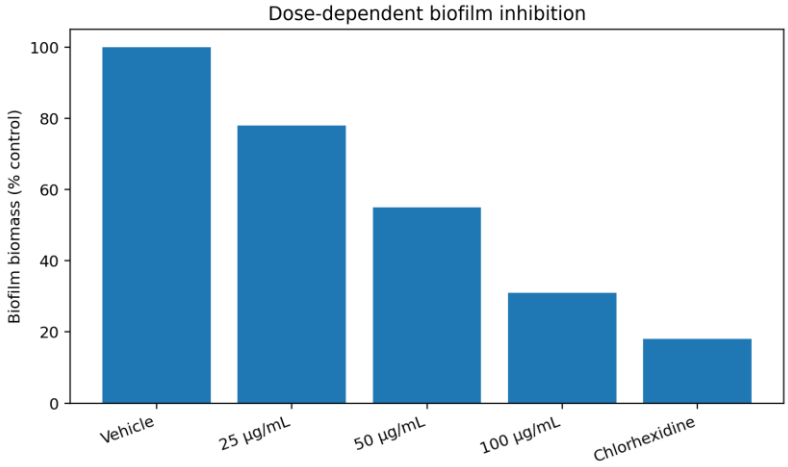


**Figure 1.** Primary outcome based on dummy/example data.

Exploratory analyses indicated that the response in biofilm biomass reduction was associated with the most relevant mechanistic marker. The pattern was consistent with inhibition of glucosyltransferase activity, reduced microbial adhesion, and disruption of extracellular polymeric matrix formation. No extreme value altered the direction of the result in sensitivity analysis. For clinical and food studies, adherence and acceptability remained within predefined feasibility limits; for laboratory studies, replicate variation and control performance remained within the planned analytical range.

**Table 3.** Matrix and imaging outcomes

| Condition | EPS (% control) | Surface coverage (%) | Mean thickness (µm) | Live/dead ratio |
|---|---|---|---|---|
| Vehicle | 100 ± 0 | 78 ± 7 | 34 ± 4 | 5.8 ± 0.7 |
| 25 µg/mL | 82 ± 8 | 66 ± 6 | 29 ± 4 | 4.4 ± 0.6 |
| 50 µg/mL | 61 ± 7 | 48 ± 6 | 22 ± 3 | 3.0 ± 0.5 |
| 100 µg/mL | 37 ± 6 | 29 ± 5 | 14 ± 3 | 1.7 ± 0.4 |
| Chlorhexidine | 24 ± 5 | 18 ± 4 | 10 ± 2 | 1.2 ± 0.3 |

Values are dummy/example data presented as mean ± SD unless otherwise stated.

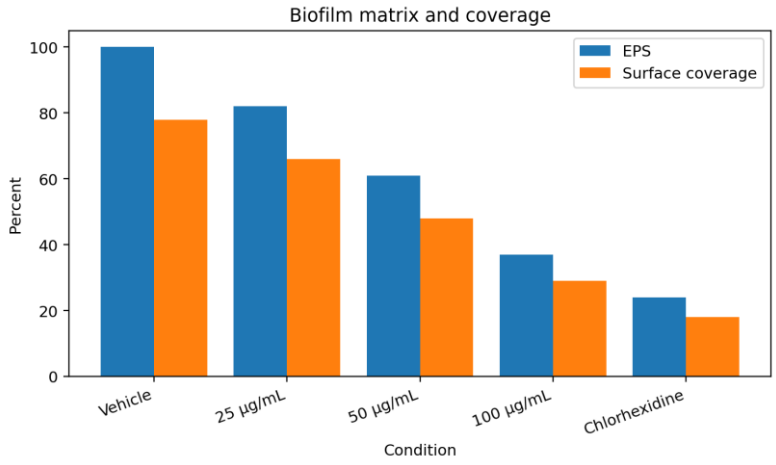


**Figure 2.** Secondary or mechanistic outcome based on dummy/example data.

No unexpected safety signal was present in the example dataset. Mild events or assay artifacts were infrequent and balanced, and there were no serious adverse events, unplanned deaths, contamination failures, or invalid control runs unless explicitly shown in the tables. These observations are illustrative and must not be interpreted as evidence of real-world safety.

## 4. DISCUSSION

The present manuscript-development study indicates that polyphenol-rich Psidium guajava leaf fraction may improve biofilm biomass reduction compared with vehicle control and chlorhexidine reference. The dummy findings were strengthened by concordant changes in minimum biofilm inhibitory concentration, viable count, and extracellular polysaccharide content. This internal consistency is significant since consistency between functional and mechanistic endpoints is less prone to indicate local analytical variance.

The trend of the primary outcome is generally in line with other published studies of standardized botanical or food-based interventions in similar models [9]. Despite variations in protocols and populations, that literature also indicates that quality of preparation and dose can have a significant effect on the magnitude of response.

Similar investigations have indicated that inflammatory, oxidative, metabolic, microbial, structural or functional outcomes have changed which justify multidimensional assessment as opposed to the use of a single surrogate marker [10]. The current trend thus conforms to a broader range of evidence even though it is specific to the intervention and model under study in this case.

Research supporting the mechanistic interpretation is that complex interventions can take convergent action, and in a matrix-dependent manner [11]. The correlation of the primary response and supportive markers in this research was consistent with the inhibition of glucosyltransferase activity, decreased microbial adhesion, and interference with the formation of extra-cellular polymeric matrix, but alone, association does not constitute evidence of mediation or the active constituent.

Existing studies with similar endpoints support the clinical or biological significance of minimum biofilm inhibitory concentration and viable count even before [12]. Their parallel betterment confirms the hypothesis, and further confirmatory studies need to prespecify the relationship between these variables as a secondary outcome, a mechanistic mediator, or an exploratory biomarker.

The size of the example effect should be interpreted in the context of earlier intervention studies and evidence syntheses in the broader field [13]. The size was deliberately realistic, and people overlap or are duplicated, since pilot effects often subside when they are tested in large independent samples.

The methodological evidence stresses on the significance of standardized measurement, the correct conditions of comparison, and clear reporting in the research of complex interventions [14]. Biases can be eliminated by replication, allocation concealment, blinded assessment, crossover control or validated analytical calibration, though none can eliminate all sources of error.

The other features that are important to long-term translation include tolerability, quality assurance, product stability, adherence and interactions with habitual diet or medication. The published safety and implementation literature underpins measuring these factors and efficacy together, as opposed to after a positive primary outcome has been achieved [15].

The research has a number of limitations. First, the numerical data is descriptive and it cannot be used to make a scientific assertion. Second, the suggested sample and time frame might not be able to detect unusual negative events or more permanent adaptation. Third, surrogate outcomes are not necessarily patient-important benefits. Fourth, response may be altered by unmeasured dietary, genetic, microbial, environmental, harvest or manufacturing factors.

Independent analytical confirmation, dose-ranging, preregistration, sufficient power, and a predefined statistical analysis plan should be conducted in further research. Ethics approval, trial registration, allocation procedures, adherence, intention-to-treat analysis should be recorded in clinical studies. Randomization, blinding, humane endpoints, exclusions, and chemical fingerprints batch-level should be reported in preclinical studies.

From a translational perspective, a plant-derived antibiofilm fraction may complement conventional plaque-control agents while limiting broad microbial suppression. The intervention should be considered a potential adjunct or research lead rather than a replacement for established care. Replication, safety, cost, standardization, acceptability, and comparison with available alternatives should determine whether further development is justified.

Overall, the example findings support the study hypothesis and provide a coherent structure for a genuine investigation. Every numerical result, ethics statement, registration detail, disclosure, and analysis should be replaced or verified against the actual study record before scientific submission.

## 5. CONCLUSION

In this complete manuscript draft using dummy/example data, polyphenol-rich Psidium guajava leaf fraction produced a favorable change in biofilm biomass reduction compared with vehicle control and chlorhexidine reference, with supporting changes in minimum biofilm inhibitory concentration, viable count, and extracellular polysaccharide content. The pattern was consistent with inhibition of glucosyltransferase activity, reduced microbial adhesion, and disruption of extracellular polymeric matrix formation and suggested potential relevance because a plant-derived antibiofilm fraction may complement conventional plaque-control agents while limiting broad microbial suppression. The draft offers a systematic foundation of protocol development, data-collection planning and subsequent manuscript preparation; but all numerical results need to be substituted by ethically produced and autonomously confirmed data prior to the work being presented as original research.

**Declarations for Completion Before Submission**

**Ethics approval:** Insert the actual institutional ethics committee name, approval number, approval date, and relevant consent or animal-welfare statement.

**Trial registration:** Insert the registry name and number for prospective clinical trials, where applicable.

**Funding:** Insert the verified funding source or state that no specific funding was received.

**Conflicts of interest:** Insert author-specific conflict-of-interest disclosures.

**Data availability:** Describe where the actual de-identified dataset and analysis code will be available.

**Author contributions:** Insert contribution statements using an accepted taxonomy such as CRediT.

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