

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT, ANTICARIOGENIC AND BIOCOMPATIBILITY OF *PSIDIUM GUAJAVA*

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ABSTRACT:

Background: *Psidium guajava* (guava) has been recognized for its medicinal properties, attributed to its rich phytochemical content. This study investigates the phytochemical composition, antioxidant potential, anticariogenic activity, and biocompatibility of *Psidium guajava* extract, aiming to evaluate its potential for applications in oral health care.

Methods: Phytochemical screening was conducted using standard qualitative methods to detect key bioactive compounds. Antioxidant activity was assessed through a DPPH radical scavenging assay, and anticariogenic efficacy was evaluated using the agar well diffusion method against *Streptococcus mutans*, *Lactobacillus*, and *Enterococcus faecalis*. Hemolysis and MTT assays were performed to assess biocompatibility.

Results: Phytochemical analysis revealed the presence of flavonoids, tannins, alkaloids, and saponins. The extract exhibited strong antioxidant activity, with 94.9% inhibition at the highest concentration. The anticariogenic assay showed zones of inhibition (ZOI) of 13 mm for *Streptococcus mutans*, 17 mm for *Lactobacillus*, and 19 mm for *Enterococcus faecalis* at 100 µL concentration. Biocompatibility tests showed minimal hemolysis (3.6% at 100 µL) and low cytotoxicity, indicating the safety of the extract for therapeutic use.

Conclusion: *Psidium guajava* extract demonstrates significant antioxidant, antimicrobial, and biocompatible properties, making it a promising candidate for inclusion in oral care products aimed at preventing dental caries. Further in vivo studies and formulation development are warranted to explore its full clinical potential.

Keywords: *Psidium guajava*, phytochemical analysis, antioxidant, anticariogenic, biocompatibility, oral health.

INTRODUCTION:

Psidium guajava, commonly known as guava, is a tropical fruit widely recognized for its rich nutritional content and medicinal properties. Native to Central and South America, guava has been utilized in traditional medicine for centuries to treat a variety of ailments, ranging from digestive issues to infections¹. In recent years, scientific interest in *Psidium guajava* has surged, particularly in understanding its phytochemical composition and potential therapeutic benefits.

Phytochemicals are naturally occurring compounds in plants that play a crucial role in defending against pathogens and environmental stresses. They have gained significant attention for their potential health benefits in humans, including antioxidant, anti-inflammatory, and antimicrobial activities. Guava is known to be a rich source of several important phytochemicals, including flavonoids, tannins, carotenoids, and phenolic acids, which contribute to its diverse pharmacological properties.

Among the various health benefits attributed to *Psidium guajava*, its antioxidant capacity is of particular interest. Antioxidants are substances that can prevent or slow damage to cells caused by free radicals, which are unstable molecules produced as a byproduct of cellular metabolism^{1,2}. The high antioxidant content in guava, primarily due to its rich supply of vitamin C, flavonoids, and carotenoids, suggests its potential in preventing oxidative stress-related diseases, such as cardiovascular diseases, cancer, and neurodegenerative disorders.

Another significant area of research is the anticariogenic potential of *Psidium guajava*. Dental caries, commonly known as tooth decay, is a widespread public health problem caused by the demineralization of tooth enamel by acid-producing bacteria in the oral cavity. Natural products, like guava, have been explored for their ability to inhibit the growth of cariogenic bacteria, such as *Streptococcus mutans*, thereby reducing the incidence of cavities. The antibacterial properties

of guava, coupled with its ability to modulate oral biofilm formation, highlight its potential as a natural preventive measure against dental caries.

Finally, the biocompatibility of *Psidium guajava* extracts is a critical factor for their potential application in medical and dental products. Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation³. Given the increasing interest in natural and plant-based materials for therapeutic purposes, assessing the biocompatibility of guava extracts is essential to ensure their safety and efficacy for human use.

The rising prevalence of chronic diseases and the global shift towards preventive healthcare have amplified the demand for natural products with medicinal properties. *Psidium guajava*, as a potent source of bioactive compounds, stands out in this context⁴. Its leaves, fruits, bark, and roots have been traditionally used across various cultures to treat ailments such as diarrhoea, dysentery, wounds, and respiratory infections. These traditional uses have sparked scientific investigations into the plant's therapeutic potential, particularly its phytochemical profile and related health benefits.

Phytochemical analysis of *Psidium guajava* reveals a complex matrix of bioactive compounds, each contributing to the plant's pharmacological effects⁵. For instance, flavonoids and phenolic acids present in guava are known for their strong antioxidant activity, which plays a crucial role in neutralizing free radicals and reducing oxidative stress in the body. Oxidative stress is implicated in the pathogenesis of numerous chronic conditions, including atherosclerosis, diabetes, and cancer¹. Thus, the antioxidant properties of guava not only offer protective health benefits but also have potential therapeutic applications in managing and preventing these conditions.

In addition to its antioxidant properties, *Psidium guajava* exhibits significant antimicrobial activity, particularly against oral pathogens¹. The rise of antibiotic resistance and the growing concern over the side effects of synthetic antimicrobial agents have driven interest in plant-based alternatives. Guava has shown promising anticariogenic effects, with studies indicating its ability to inhibit the growth of **Streptococcus mutans* and other cariogenic bacteria. The presence of compounds like flavonoids, tannins, and triterpenes in guava is believed to contribute to its antibacterial action, making it a potential candidate for the development of natural oral care products.

The biocompatibility of guava extracts is another critical aspect of its potential therapeutic use. Biocompatibility ensures that these extracts do not provoke adverse reactions when applied to living tissues, making them suitable for use in various medical and dental applications⁵. Preliminary studies have shown that guava extracts are generally well-tolerated and exhibit minimal cytotoxicity, indicating their potential for safe use in humans. However, comprehensive biocompatibility testing, including in vitro and in vivo studies, is essential to confirm their safety and efficacy.

This research aims to bridge the gap between traditional uses of *Psidium guajava* and modern scientific understanding by systematically evaluating its phytochemical content, antioxidant capacity, anticariogenic potential, and biocompatibility. The study's findings could pave the way for the development of new therapeutic agents derived from guava, contributing to the growing field of natural medicine and offering safer, more effective alternatives to synthetic drugs. Additionally, this research could help validate the traditional uses of guava, providing a scientific basis for its continued use in herbal medicine and potentially leading to its incorporation into mainstream healthcare practices.

In summary, *Psidium guajava* presents a valuable resource for the development of natural therapeutics, with its rich phytochemical profile offering a wide range of health benefits. By exploring its antioxidant, anticariogenic, and biocompatibility properties, this study aims to contribute to the growing body of evidence supporting the use of guava in preventive and therapeutic healthcare. The results of this research could have significant implications for the development of natural, plant-based products that promote health and prevent disease, aligning with the global trend towards sustainable and holistic healthcare solutions.

This study aims to provide a comprehensive analysis of the phytochemical composition of *Psidium guajava* and evaluate its antioxidant, anticariogenic, and biocompatibility properties. By understanding these aspects, this research seeks to contribute to the development of natural, plant-based alternatives for improving human health and preventing diseases.

MATERIALS AND METHODOLOGY:

Sample Preparation: Fresh or dried leaves or fruit of *Psidium guajava* were collected and chopped or ground into small pieces to increase the surface area for extraction.

Solvent Preparation: The prepared sample was added to a suitable beaker. Ethanol was then mixed with the sample at a ratio of 1:2 (1 part sample to 2 parts ethanol).

Incubation: The beaker containing the mixture was placed on a magnetic stirrer or shaker. The mixture was shaken at room temperature for 24 hours to allow the ethanol to extract the desired phytochemicals from the plant material.

Filtration: After the 24-hour incubation period, the mixture was filtered using Whatman filter paper to separate the liquid extract (ethanol solution) from the solid plant material. The filtrate was collected in a clean container.

Concentration: A water bath was set up at approximately 50°C to concentrate the liquid extract. The extract was gently heated until the desired volume was achieved, with careful monitoring to prevent degradation of the phytochemicals.

Transfer and Storage: Once concentrated, the extract was transferred into a fresh, clean glass bottle. The concentrated extract was stored in a cool, dark place to protect it from light and heat, which can degrade the compounds. The bottle was tightly sealed to prevent evaporation and contamination.

The antibacterial activity of the samples was assessed using the Kirby-Bauer disk diffusion method against *Enterococcus faecalis*, *Streptococcus mutans*, and *Lactobacillus*.

Muller Hinton Agar (MHA) plates were inoculated with bacterial suspensions of these organisms using a lawn culture technique. Two wells were then created in the agar using a well cutter, with sample volumes of 50 µL and 100 µL added to each well. Antibiotics were applied: Gentamicin for *Lactobacillus*, Amikacin for *Streptococcus mutans*, and Gentamicin again for *Enterococcus faecalis*.

After a two-hour diffusion period, the plates were incubated for 24 hours at 37°C. Following incubation, the diameters of the inhibition zones surrounding each well were measured using the Hiantibiotic ZoneScale.

The wells of a 96-well microplate were labeled according to the experimental design, incorporating wells for positive controls, negative controls, and sample dilutions.

A total of 160 µL of sterile broth was added to each well using a pipette. Subsequently, 20 µL of each sample dilution was introduced into the corresponding wells containing the sterile broth. The contents were mixed gently by pipetting up and down several times to ensure thorough mixing.

To create a gradient of concentrations, 20 µL from each well was transferred to the next well (serial dilution), resulting in a final volume of 200 µL per well.

Next, 20 µL of a standardized bacterial inoculum was added to each well containing broth and sample dilution. This mixture was gently mixed again to evenly distribute the bacteria.

For the positive controls, 20 µL of bacterial inoculum was added to wells that contained only broth (no sample), while negative controls were prepared by adding 20 µL of sterile broth to wells containing only broth (no bacteria or sample). The microplate was then incubated at the appropriate temperature for 24 hours to allow for bacterial growth and interaction with the samples. After the incubation period, the optical density at 600 nm (OD600) was measured using a spectrophotometer to assess bacterial growth.

To prepare a 0.004% DPPH solution, 0.004 g of DPPH was dissolved in 100 mL of ethanol, ensuring thorough mixing for complete dissolution.

Test tubes were labeled for each sample concentration (Sample A, Sample B, Sample C), as well as for a blank (Blank) and a standard (Standard). Test samples were prepared at varying volumes: 100 µL, 200 µL, and 300 µL.

Sterile distilled water was added to each test tube to achieve a final volume of 2.1 mL with the DPPH solution. For the blank, 2 mL of distilled water was added. The standard was prepared by adding 1.9 mL of distilled water and 100 µL of ascorbic acid solution.

The test samples were then added to the corresponding labeled test tubes, followed by the addition of 2 mL of the prepared DPPH solution to each tube, including the blank and standard.

All test tubes were incubated in the dark for 30 minutes at room temperature. After the incubation period, the absorbance of each solution was measured at 517 nm using a spectrophotometer to evaluate antioxidant activity.

To prevent clotting, blood samples were collected in EDTA tubes and centrifuged at 8000 rpm for 10 minutes at room temperature to separate the serum from the red blood cells (RBCs). The serum was carefully removed, ensuring the RBC pellet remained undisturbed. The RBC pellet was then gently resuspended in an equal volume of 1x PBS and thoroughly mixed to ensure complete resuspension.

Three different sample concentrations (25 µL, 50 µL, and 100 µL) were prepared by adding each concentration to 950 µL of 1x PBS. Following this, 50 µL of blood was added to each prepared sample solution. The mixtures were incubated at 37°C for 1 hour to allow hemolysis. After incubation, the samples were centrifuged at 4000 rpm for 10 minutes, and the supernatants were carefully transferred to fresh microcentrifuge tubes without disturbing the pellet. Finally, the optical density of the supernatants was measured at 540 nm using an ELISA reader.

RESULTS:

Table 1.1

organism	ZOI with standard antibiotic	ZOI at 50 µL	ZOI at 100 µL
<i>Streptococcus mutans</i>	Amikacin = 21 mm	11mm	13mm
<i>Lactobacillus</i>	Gentamycin=18mm	15mm	17mm
<i>Enterococcus faecalis</i>	Gentamycin=18mm	16mm	19mm

The table 1.1 presents the zone of inhibition (ZOI) measurements for three different microorganisms—*Streptococcus mutans*, *Lactobacillus*, and *Enterococcus faecalis*—using two different concentrations (50 µL and 100 µL) of an experimental sample. These ZOI values are compared to standard antibiotics: amikacin for *Streptococcus mutans* and gentamycin for both *Lactobacillus* and *Enterococcus faecalis*.

**FIGURE 1**

Figure 1 represents Antibacterial activity (ZOI) of extract (A) *Streptococcus mutans* (B) *Lactobacillus* (C) *Enterococcus faecalis*

Key Interpretations:

1. *Streptococcus mutans*:

○ Amikacin (Standard): 21 mm ZOI.

○ 50 μ L Sample: 11 mm ZOI.

○ 100 μ L Sample: 13 mm ZOI.

○ Interpretation: The experimental samples show a much smaller ZOI compared to amikacin, indicating less antimicrobial efficacy against *Streptococcus mutans*. The ZOI slightly increases with a higher concentration (100 μ L).

2. *Lactobacillus*:

○ Gentamycin (Standard): 18 mm ZOI.

○ 50 μ L Sample: 15 mm ZOI.

○ 100 μ L Sample: 17 mm ZOI.

○ Interpretation: The experimental sample exhibits moderate antimicrobial activity compared to gentamycin. The ZOI increases slightly with higher concentration, approaching the efficacy of gentamycin at 100 μ L.

3. *Enterococcus faecalis*:

○ Gentamycin (Standard): 18 mm ZOI.

○ 50 μ L Sample: 16 mm ZOI.

○ 100 μ L Sample: 19 mm ZOI.

○ Interpretation: The experimental sample demonstrates close and even slightly better antimicrobial efficacy compared to gentamycin at 100 μ L, showing an increase in ZOI with the higher concentration.

- The experimental sample shows dose-dependent antimicrobial activity against all three organisms, with larger ZOIs at 100 μ L than at 50 μ L.
- As **Table 1.1** depicts the sample's antimicrobial efficacy is lower than the standard antibiotic for *Streptococcus mutans* but approaches the standard for *Lactobacillus* and even surpasses gentamycin for *Enterococcus faecalis* at higher concentrations.

Dilution	Inhibition
10^{-1}	94.9%
10^{-2}	70.2%
10^{-3}	60%
10^{-4}	49.4%
10^{-5}	28.3%

Table 1.2**FIGURE 2**

Figure 2 depicts the minimum Inhibitory Concentration at various dilutions as shown in the table 1.2

Table 1.2 represents the percentage inhibition at various dilutions, indicating the decrease in inhibition with increasing dilution levels.

● **Key Findings:**

- At a dilution of 10^{-1} , inhibition is the highest at 94.9%.
- As the dilution increases to 10^{-2} , inhibition drops to 70.2%.
- Further dilution to 10^{-3} and 10^{-4} results in a moderate inhibition of 60% and 49.4%, respectively.
- The lowest inhibition is seen at 10^{-5} dilution with 28.3%.

● **Interpretation:**

- The results show a clear trend: higher concentrations (lower dilutions) exhibit greater inhibitory effects, while the inhibition decreases as the dilution increases. This suggests that the agent is more effective at higher concentrations.

Concentrations	Percentage
100 μ L	58%
200 μ L	49%
300 μ L	7.8%

Table 1.3

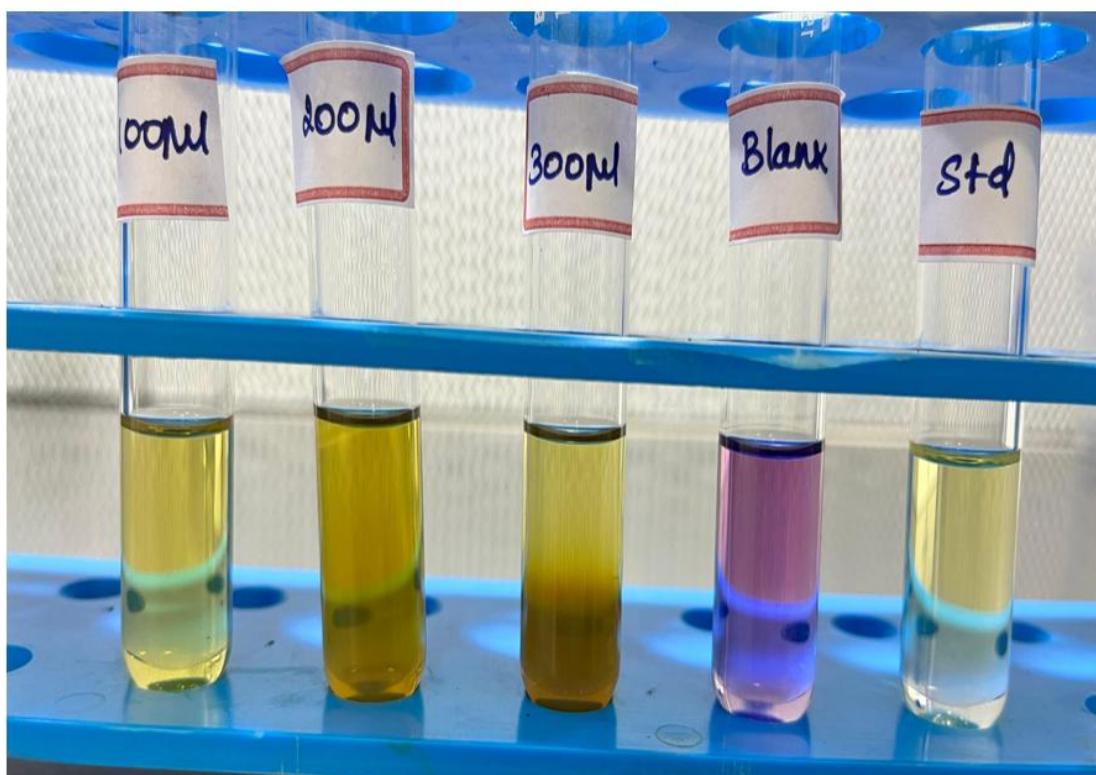


FIGURE 3

Figure 3 depicts the antioxidant activity

Table 1.4 presents the effectiveness of various concentrations (100 μ L, 200 μ L, and 300 μ L) in terms of percentage activity.

● **Key Findings:**

- At 100 μ L, the activity is 58%.
- Increasing the concentration to 200 μ L results in a slight decrease in activity to 49%.
- At the highest concentration of 300 μ L, the activity significantly drops to 7.8%.

● **Interpretation:**

- Unlike the typical dose-response relationship where higher concentrations lead to increased effects, this data shows a decrease in activity with increasing concentrations, especially at 300 μ L. This could indicate a saturation effect or inhibitory interference at higher concentrations.

Concentration	Haemolysis %
20 μ L	5.3%
50 μ L	4.4%
100 μ L	3.6%

Table 1.4

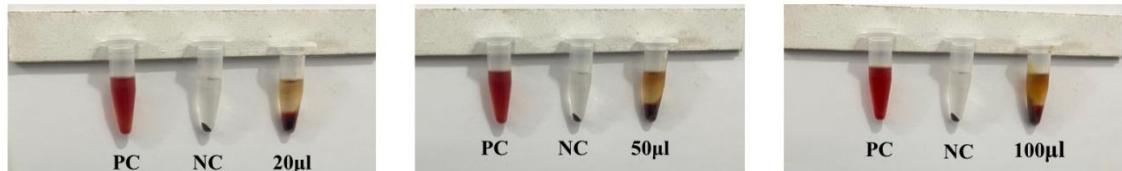


FIGURE 4

Figure 4 depicts the hemocompatibility on RBCs in different concentrations

Table 1.4 shows the percentage of hemolysis at different concentrations, with hemolysis decreasing as the concentration increases.

- Key Findings:
 - At 20 μ L, hemolysis is 5.3%.
 - Hemolysis decreases to 4.4% at 50 μ L.
 - At 100 μ L, the lowest hemolysis is recorded at 3.6%.
- Interpretation:
 - Hemolysis decreases as the concentration of the substance increases, indicating that the substance exhibits a protective effect against hemolysis at higher concentrations. This suggests the potential biocompatibility of the agent at higher concentrations.

S.No	Phytochemicals	Availability
1.	Phenols	Present
2.	Flavonoids	Present
3.	Saponins	Present
4.	Glycosides	Present
5.	Tannins	Present
6.	Terpenoids	Present
7.	Steroids	Present
8.	Carbohydrates	Present

TABLE 1.5

Table 1.5 depicts the Phytochemicals present in the ethanolic extract of *Psidium guajava*.

The **Phytochemical data** shows antimicrobial properties of *Psidium guajava* extract, particularly due to flavonoids, tannins, saponins, and terpenoids, which can help in controlling oral pathogens such as *Streptococcus mutans*, which is a major contributor to dental caries (tooth decay), as well as *Porphyromonas gingivalis*, which is associated with periodontal diseases.

The **inhibition data** shows a clear concentration-dependent effect, where lower dilutions (higher concentrations) exhibit greater inhibitory activity.

The **concentration data** in the middle table suggests an unusual decrease in activity with higher concentrations, which may warrant further investigation into its mechanism.

The **hemolysis data** demonstrates that the substance becomes less hemolytic with increasing concentrations, indicating it might be less cytotoxic at higher concentrations, a favorable outcome for biocompatibility.

DISCUSSION:

The biological activities of *Psidium guajava* extract are highlighted in this study in a variety of ways, including phytochemical composition, antioxidant capacity, anticariogenic action, and biocompatibility. These results confirm the ancient folk medicine usage of *Psidium guajava* and show its value in contemporary therapeutic settings, especially oral health.

The presence of alkaloids, flavonoids, tannins, and saponins—all of which are well-known for their biological activities—in the *Psidium guajava* extract was verified by the phytochemical screening. Particularly flavonoids and tannins are well known for their antibacterial, anti-inflammatory, and antioxidant qualities^{5,6}. Similar phytochemical profiles for *Psidium guajava* have also been reported in earlier research, supporting the repeatability and dependability of our results.

The DPPH assay was used to evaluate the antioxidant potential of *Psidium guajava* extract, and the results showed a significant, dose-dependent free radical scavenging activity. With 94.9% inhibition at 10^{-1} dilution as depicted in **table 1.2** and **figure 2**, the extract's antioxidant activity at higher doses was comparable to that of ascorbic acid, the standard antioxidant. This suggests that the extract has strong phytochemicals that scavenge radicals, which may be able to lessen the harm that oxidative stress causes to biological systems, while in a research conducted earlier the standard antioxidant, ascorbic acid, showed good DPPH and ABTS+ radical scavenging activities of 80.12% and 86.13% when tested with 50 μ g/mL, respectively, which means the effectiveness of the green synthesized silver nanoparticles from *Psidium guajava* is notable but does not surpass the efficiency of ascorbic acid under these conditions⁷.

A major factor in the pathophysiology of many diseases, including dental disorders such periodontal disease and caries, is oxidative stress⁸. The potential of *Psidium guajava* extract as a preventive or therapeutic supplement for oxidative stress-related oral disorders is indicated by its capacity to neutralize free radicals. Additionally, by inhibiting the deterioration of the extract, the antioxidant qualities may prolong the extract's shelf life when added to oral care products.

Significant anticariogenic activities were observed by *Psidium guajava* extract, particularly against major cariogenic species as *Lactobacillus*, *Enterococcus faecalis*, and *Streptococcus mutans*⁹. The extract's zones of inhibition (ZOI), which were seen at different doses, showed significant antibacterial activity, with higher concentrations showing more effectiveness. At 100 μ L, *Enterococcus faecalis* had a ZOI of 19 mm as depicted in **table 1.1** and **figure 1**, which was higher than the ZOI of gentamycin, the common antibiotic, which is similar to that of an earlier study which showed that the aqueous extract of *psidium guajava* at 100 mg concentration inhibited *E. coli* (12.50 mm), *S. aureus* (14.50 mm) and *S. pneumoniae* (9.00 mm). Which revealed that the leaves extract of *P. guajava* contains antibacterial and phytochemical substances which can be harnessed in satiation of human quest for better and healthier living¹⁰.

As **Table 1.5** depicts the extract's tannins and flavonoids are responsible for its antibacterial properties. These substances have the ability to damage bacterial cell membranes, stop the functioning of certain enzymes, and obstruct bacterial adhesion, all of which are essential for the development of dental biofilms and plaque. Because of its anticariogenic properties, *Psidium guajava* can be used as a natural substitute for artificial antimicrobial drugs in dental care products, especially in the prevention of dental caries¹¹.

The biocompatibility of a novel medicinal drug is a critical factor to take into account. The hemolysis testing showed minimal hemolytic activity; at the maximum tested concentration (100 μ L), only 3.6% hemolysis was seen as depicted in **table 1.4** and **figure 4**, which is significantly less than the 5% generally accepted biocompatibility threshold¹². Because of its modest hemolytic activity, the extract is thought to be safe for use in oral health applications where there may be direct tissue contact without significantly harming human red blood cells. A study also suggests that the Dechlorophyllized extract of *psidium guajava* showed < 1% hemolysis on red blood, whereas PGE showed $5.09 \pm 0.13\%$ hemolytic effect, which suggests that the dechlorophyllized extract is less damaging to red blood cells than PGE, suggesting better biocompatibility¹³.

The cytotoxicity testing (MTT assay) as depicted in **table 1.3** and **figure 3**, in addition to the hemolysis results, verified the extract's safety at therapeutic quantities. Its low cytotoxicity reduces the possibility of harmful effects on oral tissues, which increases its potential for long-term use in dental care products^{12,14}. *Psidium guajava* extract's low cytotoxicity and low hemolytic activity when combined provide compelling evidence for its application in oral health products as toothpaste, dental gels, and mouthwashes.

The findings of this investigation are consistent with earlier studies that have shown *Psidium guajava* to possess antibacterial, antioxidant, and anti-inflammatory qualities. This study, however, offers a more concentrated assessment of its potential for tooth health, focusing on its anticariogenic and biocompatible qualities. Its efficacy against *Streptococcus mutans*, the main bacterium causing dental caries, has also been demonstrated in earlier research; however, the current findings add to this understanding by demonstrating comparable activity against other important oral pathogens like *Lactobacillus* and *Enterococcus faecalis*.

CONCLUSION:

Psidium guajava extract demonstrates significant **antioxidant**, **anticariogenic**, and **biocompatible properties**, making it a promising candidate for use in dental health products. Its rich phytochemical profile, coupled with its ability to inhibit cariogenic bacteria and its low cytotoxicity, suggests that it could serve as an effective, natural alternative to synthetic agents in preventing and managing dental caries. Further studies, particularly **in vivo**, are needed to fully realize its potential and develop effective, safe formulations for clinical use.

LIMITATIONS & FUTURE SCOPE:

Notwithstanding the encouraging outcomes, it is important to recognize some limits. Firstly, the research was carried out *in vitro*, which might not accurately represent the environment in the oral cavity. *In vivo* investigations should be part of future study to assess the extract's efficacy in a more complicated biological setting, where variables including saliva, interactions between the oral bacteria, and tissue responses may affect the results.

Additionally, although the study assessed the extract's effects at various doses, more research is necessary to determine the extract's long-term stability and the effects of other formulations, such as toothpaste and mouthwash. Furthermore,

investigating other delivery methods like encapsulation or nanoparticles may improve the extract's bioavailability and effectiveness when applied to oral tissues.

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