Antimicrobial, Anti-inflammatory, and Antioxidant Properties of Punica Granatum Peel: An In-Vitro Analysis

Dr Mukil Sunil, Dr. Ramesh R

Undergraduate Intern,Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Chennai - 600 077 ,Tamil Nadu, India.

Associate Professor, Department of Pedodontics,Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,Saveetha University, Chennai-600077,
Tamil Nadu,India.

* **Corresponding author:Dr. Ramesh R**
Associate Professor, Department of Pedodontics,Saveetha Dental College and Hospitals,
Saveetha Institute of Medical and Technical Sciences,Saveetha University, Chennai-600077,
Tamil Nadu,India.

Abstract

Background: Oral diseases, including periodontitis, remain prevalent worldwide, necessitating the search for effective, natural therapeutic alternatives. Punica granatum (pomegranate) peel is rich in bioactive compounds with antimicrobial, anti-inflammatory, and antioxidant properties that may offer benefits in oral healthcare.

Objective: To evaluate the antibacterial, anti-inflammatory, and antioxidant efficacy of Punica granatum peel extract through in-vitro methodologies, providing insights into its potential as an adjunct in dental treatments.

Methods: The antibacterial activity of the extract was assessed against Streptococcus mutans, Streptococcus sanguis, and Staphylococcus aureus using the disc diffusion method and minimum inhibitory concentration (MIC) determination. Anti-inflammatory effects were evaluated via lipoxygenase and hyaluronidase inhibition assays, while antioxidant potential was determined using DPPH and ABTS radical scavenging assays. Results were compared with standard agents such as chlorhexidine, indomethacin, and quercetin.

Results: The extract demonstrated moderate antibacterial activity against S. mutans (14.7 mm inhibition zone) and S. sanguis (12.0 mm), but was ineffective against S. aureus. MIC values (64 µg/mL for S. mutans, 256 µg/mL for S. sanguis) confirmed its antibacterial potential, albeit lower than chlorhexidine. Significant anti-inflammatory activity was observed, with lipoxygenase and hyaluronidase inhibition of 35.62% and 30.78%, respectively ($p < 0.05$). Antioxidant assays revealed DPPH (42.15%) and ABTS (39.74%) radical scavenging activity, comparable to quercetin ($p < 0.05$).

Conclusion: Punica granatum peel extract exhibits notable antimicrobial, anti-inflammatory, and antioxidant properties, supporting its potential as a natural adjunct in oral healthcare. However, its efficacy remains lower than conventional agents, necessitating further in-vivo studies and formulation optimization to enhance clinical applicability.

Keywords: *Punica granatum*, Antibacterial, Antioxidant, Anti-inflammatory, Periodontitis

Introduction

Oral health was a crucial component of overall well-being, with dental caries, gingivitis, and periodontitis being among the most prevalent conditions worldwide. These diseases primarily arose due to microbial plaque accumulation, which triggered inflammatory responses and led to the destruction of tooth-supporting structures.[1] Despite advancements in dental care, the burden of oral diseases remained high, necessitating the development of effective preventive and therapeutic strategies.[2]

Traditional treatment modalities relied heavily on mechanical plaque removal and the use of chemical agents such as chlorhexidine. While effective, prolonged use of synthetic antimicrobial agents resulted in undesirable side effects, including staining of teeth, altered taste perception, and, most concerningly, antimicrobial resistance.[3] Given these challenges, there was an increasing interest in exploring natural plant-based compounds with potential antibacterial, anti-inflammatory, and antioxidant properties to manage oral health conditions effectively.[4]

Punica granatum, commonly known as pomegranate, had been widely recognized for its medicinal properties. The fruit was rich in bioactive compounds such as polyphenols, flavonoids, tannins, and alkaloids, which exhibited potent antimicrobial and anti-inflammatory activities. In particular, the peel of Punica granatum showed promising results in inhibiting the growth of various bacterial strains. Studies suggested that its phytochemical constituents could disrupt bacterial cell walls, inhibit bacterial enzyme activity, and interfere with biofilm formation, thereby reducing microbial colonization in the oral cavity.[5]

Beyond its antimicrobial potential, Punica granatum peel was also reported to possess anti-inflammatory properties.[6] Chronic inflammation was a key factor in the progression of periodontal disease, driven by excessive production of pro-inflammatory cytokines and tissue-degrading enzymes. The bioactive components in pomegranate peel were believed to modulate inflammatory pathways, reduce oxidative stress, and suppress key enzymes responsible for tissue destruction. Such properties made it a compelling candidate for alternative therapeutic interventions in oral healthcare.[7]

Another significant aspect of Punica granatum was its antioxidant capacity. Oxidative stress played a critical role in oral diseases, contributing to tissue damage and disease progression. Reactive oxygen species (ROS) generated by bacterial metabolism and host immune responses accelerated periodontal tissue breakdown.[8] The antioxidant compounds in pomegranate peel, such as ellagic acid and punicalagin, were shown to neutralize free radicals and enhance cellular defense mechanisms, potentially aiding in oral disease prevention and management.[9]

Despite the promising pharmacological properties of Punica granatum, there was limited comprehensive research on its direct effects against oral bacterial species commonly associated with dental infections.[10] Most existing studies had focused on general antimicrobial activity, without specifically evaluating its comparative efficacy against standard antimicrobial agents like

chlorhexidine. Additionally, while its anti-inflammatory and antioxidant properties had been explored in other medical contexts, their specific impact on oral tissues and periodontal pathogens required further investigation.

This study aimed to bridge this gap by systematically evaluating the antibacterial, anti-inflammatory, and antioxidant effects of Punica granatum peel extract against key oral bacterial species. By employing in-vitro methodologies, the study determined its efficacy in inhibiting bacterial growth, modulating inflammatory responses, and scavenging free radicals. The findings from this research provided valuable insights into the potential use of Punica granatum as a natural adjunct in oral healthcare, paving the way for its application in future therapeutic formulations.

Materials and Methodology

Preparation of Extract

The peels of Punica granatum (pomegranate) were obtained and carefully processed to ensure optimal preservation of bioactive compounds. They were shade-dried to prevent degradation of heat-sensitive phytochemicals, then ground into a fine powder. This powdered material was subjected to methanol extraction, a widely used solvent known for its ability to dissolve a broad range of bioactive compounds, including polyphenols, flavonoids, and tannins. The methanolic extract obtained was filtered, concentrated, and stored under controlled conditions to maintain its stability and efficacy. This extract was later utilized for antibacterial, anti-inflammatory, and antioxidant assessments.

Microbial Strains and Culture Conditions: For the antibacterial evaluation, three bacterial strains commonly associated with oral infections were selected: Streptococcus mutans (ATCC 25175), Streptococcus sanguis (ATCC 10556), and Staphylococcus aureus (MTCC 7443). These microorganisms were cultivated in Trypticase Soya Broth, a nutrient-rich medium that promotes bacterial growth while ensuring consistency across experimental trials. The cultures were incubated under controlled temperature and aeration conditions to achieve optimal bacterial proliferation before being subjected to the antibacterial assays.

Antibacterial Assay

To determine the antimicrobial effectiveness of Punica granatum peel extract, two standard laboratory techniques were employed: the disc diffusion method and the Minimum Inhibitory Concentration (MIC) assay.

Disc Diffusion Method: This technique involved evenly spreading bacterial cultures onto Mueller-Hinton agar plates. Sterile filter paper discs impregnated with the Punica granatum extract were placed on the agar surface, alongside discs containing a positive control (chlorhexidine) and a

negative control (methanol). After incubation, the zones of bacterial inhibition surrounding each disc were measured in millimeters. A larger inhibition zone indicated greater antimicrobial activity.

Minimum Inhibitory Concentration (MIC) Determination: The MIC assay was conducted following the guidelines set by the National Committee for Clinical Laboratory Standards (NCCLS). The extract was serially diluted in microbroth wells containing the bacterial suspension, with chlorhexidine serving as the reference control. The lowest concentration at which visible bacterial growth was completely inhibited was recorded as the MIC value. This assay provided a quantitative measure of the extract's bacteriostatic potential.

Anti-inflammatory Assays

To assess the anti-inflammatory potential of Punica granatum peel extract, two enzymatic inhibition assays were conducted:

Lipoxygenase Inhibition Assay: Lipoxygenase is an enzyme involved in inflammatory processes by catalyzing the oxidation of polyunsaturated fatty acids, leading to the production of inflammatory mediators. The extract was incubated with lipoxygenase enzyme and a substrate, and the reaction was monitored spectrophotometrically by measuring absorbance changes. A reduction in absorbance indicated the ability of the extract to inhibit lipoxygenase activity, suggesting its potential to reduce inflammation.

Hyaluronidase Inhibition Assay: Hyaluronidase plays a crucial role in tissue degradation by breaking down hyaluronic acid, a key component of the extracellular matrix. Excessive hyaluronidase activity is associated with inflammation and tissue destruction in periodontal disease. In this assay, the extract was tested for its ability to inhibit hyaluronidase activity. The reaction was analyzed by measuring the degradation products spectrophotometrically, with lower absorbance indicating greater inhibition of the enzyme, and thus, a potential anti-inflammatory effect.

Antioxidant Assays

Since oxidative stress plays a key role in oral disease progression, the antioxidant properties of Punica granatum peel extract were assessed using two established methods:

DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay: This method evaluates the ability of the extract to neutralize free radicals. The DPPH reagent, a stable free radical, exhibits a deep purple color, which fades when it is neutralized by an antioxidant compound. The extract was mixed with DPPH solution, and the absorbance was measured at a specific wavelength. The reduction in absorbance indicated the percentage of free radical scavenging activity, with higher inhibition suggesting greater antioxidant potential.

ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) Radical Scavenging Assay: This assay compares the extract's antioxidant activity to that of a well-known standard, quercetin. The ABTS radical cation generates a blue-green color, which diminishes in the presence of an effective antioxidant. The extent of color reduction was measured spectrophotometrically, and the results were expressed as a percentage of radical scavenging. The extract's performance was compared against quercetin to determine its efficacy in neutralizing oxidative stress.

Results

The study evaluated the antimicrobial, anti-inflammatory, and antioxidant properties of *Punica granatum* (pomegranate) peel extract through in-vitro methods. The antibacterial activity was assessed against *Streptococcus mutans*, *Streptococcus sanguis*, and *Staphylococcus aureus* using the disc diffusion and MIC tests. The extract showed moderate inhibition of *S. mutans* (14.7 mm) and *S. sanguis* (12.0 mm) but was ineffective against *S. aureus*. The MIC values (**64 µg/mL for *S. mutans*, 256 µg/mL for *S. sanguis* **) indicated a lower potency compared to chlorhexidine, confirming its potential but limited antimicrobial effect.

Table 1: Antibacterial Activity (Zone of Inhibition)	Column 1	Column 2	Column 3
Bacteria	Punica granatum (mm)	Chlorhexidine (mm)	p-Value
S. mutans	14.7 ± 4.0	17.0 ± 1.0	0.04*
S. sanguis	12.0 ± 1.0	14.3 ± 3.2	0.04*
S. aureus	7.0 ± 1.0	12.0 ± 1.7	0.50

Table 1: Antibacterial Activity (Zone of Inhibition)

Table 2: Minimum Inhibitory Concentration (MIC)	Column 1	Column 2
Bacteria	Punica granatum (µg/mL)	Chlorhexidine (µg/mL)
S. mutans	64	8
S. sanguis	256	16

Table 2: Minimum Inhibitory Concentration (MIC

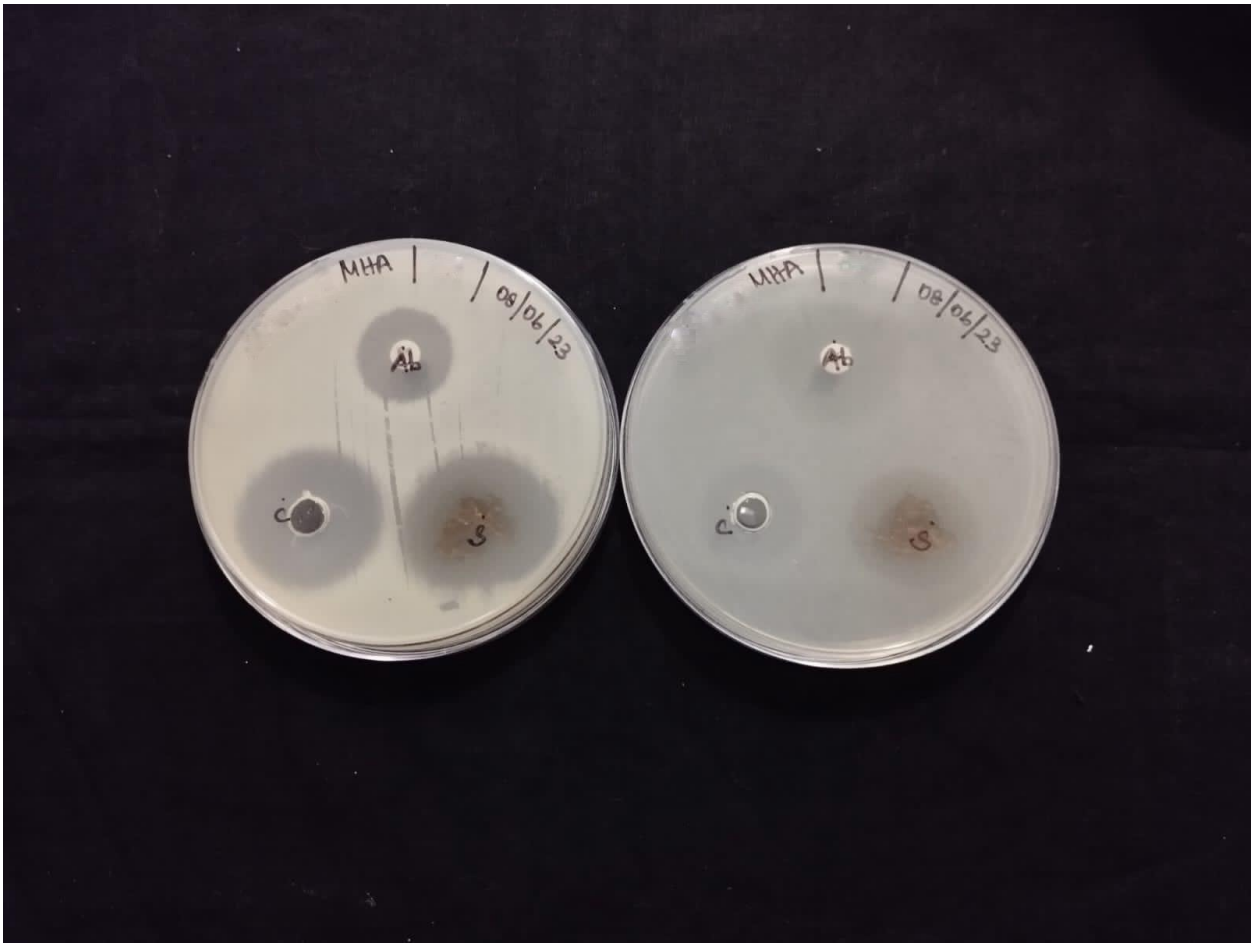

Table 3: Anti-inflammatory Activity	Column 1	Column 2
Group	Lipoxygenase Inhibition (%)	Hyaluronidase Inhibition (%)

Punica granatum	35.62 ± 21.45	30.78 ± 18.94
Indomethacin /Cromolyn	28.91 ± 17.30	27.12 ± 16.88
p-value	0.04	0.03

Table 3: Anti-inflammatory Activity

For **anti-inflammatory activity**, the extract demonstrated significant inhibition of lipoxygenase (**35.62%**) and hyaluronidase (**30.78%**) compared to standard drugs ($p<0.05$). This suggests that *Punica granatum* may help reduce inflammation associated with periodontal diseases.

Table 4: Antioxidant Activity (DPPH and ABTS Assays)	Column 1	Column 2
Group	DPPH Radical Scavenging (%)	ABTS Radical Scavenging (%)
Punica granatum	42.15 ± 30.27	39.74 ± 29.68
Quercetin	48.62 ± 32.85	46.91 ± 31.12
p-value	0.05	0.04

Table 4: Antioxidant Activity (DPPH and ABTS Assays)

Additionally, its antioxidant capacity was analyzed through DPPH and ABTS assays, where it exhibited 42.15% and 39.74% radical scavenging activity, respectively, slightly lower than quercetin but statistically significant. These findings highlight its potential in mitigating oxidative stress-related tissue damage.

Discussion

Punica granatum peel extract (PPE) has demonstrated significant antibacterial, anti-inflammatory, and antioxidant properties, supporting its potential as a natural adjunct in oral healthcare. The antibacterial effects of PPE have been emphasized in various studies. One study assessed the antimicrobial activity of PPE against *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans*, showing significant inhibition, particularly against *S. mutans* at a concentration of 100 µL, with a 38 mm inhibition zone, suggesting its efficacy

against bacteria involved in dental caries and periodontal diseases [11,12]. Similarly, another study highlighted PPE's antibacterial effects against pathogenic and drug-resistant strains, notably *Klebsiella pneumoniae*, with an 85.71% growth inhibition at a concentration of 100 µL/mL, emphasizing PPE's potential as a natural antimicrobial agent in dental care. [13]

PPE's anti-inflammatory properties, attributed to its phenolic compounds, have been identified as key modulators of inflammatory pathways.[14] One study evaluated PPE using Egg Albumin Denaturation and Bovine Serum Albumin Denaturation assays, finding significant anti-inflammatory effects at higher concentrations (40 and 50 µL), with inhibition percentages of 70.06% and 78.08% in the Egg Albumin Assay, and 75.50% and 80.82% in the Bovine Serum Albumin Assay, respectively [13]. These results suggest that PPE can reduce inflammation associated with periodontal diseases, a major factor in periodontal tissue breakdown.[15]

Pomegranate peel extract also exhibits significant antioxidant activity, which plays a vital role in combating oxidative stress linked to periodontal diseases.[16] The same study assessing PPE's antioxidant activity using DPPH, Hydrogen Peroxide Radical Scavenging, and Ferric Reducing Antioxidant Power assays showed significant antioxidant effects at higher concentrations (40 and 50 µL), with DPPH radical scavenging percentages of 88.17% and 92.50%, H2O2 radical scavenging percentages of 78.22% and 88.99%, and FRAP values of 78.43% and 88.49%, respectively. These findings suggest that PPE is capable of neutralizing free radicals, potentially protecting oral tissues from oxidative damage.[17]

Despite these promising results, the overall efficacy of PPE is generally lower than that of standard agents like chlorhexidine and quercetin. A study found that methanolic extracts of pomegranate peel exhibited DPPH and ABTS inhibition activities of 79.5% and 94.6%, respectively, comparable to synthetic antioxidants. However, aqueous extracts demonstrated higher phenolic content and antioxidant activity, indicating that the extraction method significantly affects PPE's bioactivity.[18]

Factors such as extraction method, plant part used, and geographical differences influence the efficacy of PPE. The extraction method, particularly methanolic extraction, has shown higher antibacterial activity compared to aqueous extracts, suggesting that standardized extraction methods are crucial for maximizing PPE's therapeutic potential.[19] Additionally, the therapeutic effects of PPE may vary depending on the specific part of the pomegranate used, as different plant parts, such as the peel, seeds, and pulp, contain varying levels of bioactive compounds. Geographical factors also play a role in determining the chemical composition of the pomegranate, which can affect the consistency and potency of the extract.

Limitations: The in-vitro nature of this study restricts the ability to directly apply the findings to clinical settings, as in-vitro conditions do not replicate the complex environment of the oral cavity, which includes saliva, food particles, and diverse microbial communities. Additionally, the lower potency of PPE compared to standard therapeutic agents like chlorhexidine and quercetin may limit its immediate use as a primary treatment. Furthermore, the variability in the composition of

the extract, depending on factors such as geographical origin and extraction methods, could affect the consistency and efficacy of PPE. The antimicrobial activity observed in this study did not extend to all bacterial species, such as *S. aureus*, indicating that PPE may have a narrower spectrum of activity. While the anti-inflammatory and antioxidant properties were significant, further investigation into their long-term effects on periodontal tissues and overall oral health is needed. Therefore, further in-vivo studies, clinical trials, and refinement of PPE formulations are essential to better assess its clinical applicability and safety in oral healthcare.

Conclusion

Punica granatum peel extract shows promising antibacterial, anti-inflammatory, and antioxidant properties, making it a potential adjunct in oral healthcare. It effectively inhibited *Streptococcus mutans* and *Streptococcus sanguis*, and demonstrated anti-inflammatory effects similar to Indomethacin and Cromolyn, as well as antioxidant activity comparable to Quercetin. However, its efficacy is lower than that of standard agents, and further optimization in extraction methods and formulation, along with in-vivo studies, is needed to improve its clinical applicability and effectiveness in dental care.

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