Synthesis of Hyaluronic Acid/pine Extract Gel as local drug delivery for Chronic Periodontitis

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Abstract

Introduction: Pine bark extract is a widely recognized herbal supplement known for its antimicrobial, antioxidant, and anti-inflammatory properties. Hyaluronic acid, a well-established biomaterial, has shown promising applications in periodontal therapy. This study aims to evaluate the synergistic effects of combining pine bark extract with hyaluronic acid, exploring its potential as a novel therapeutic agent for periodontal treatment.

Aim: The objective of this study is to assess the combined effects and properties of pine bark extract when formulated with hyaluronic acid gel for use as a local drug delivery agent.

Materials and Methods: A gel formulation was prepared by combining hyaluronic acid and pine bark extract in specific ratios. The antioxidant properties were evaluated using the DPPH assay, while anti-inflammatory activity was assessed through a protein denaturation assay. The spreadability of the prepared gel was determined using the glass slide method to measure its applicability.

Results: The study demonstrated that increasing the concentration of the pine bark extract-hyaluronic acid complex resulted in a proportional enhancement of its antioxidant and anti-inflammatory effects. The spreadability of the prepared gel was recorded at 32 mm and 34 mm in microbial plate tests, indicating its suitability for clinical application.

Conclusion: The findings suggest that the novel hyaluronic acid-pine bark extract gel exhibits significant antioxidant and anti-inflammatory properties, along with favorable spreadability. These attributes indicate its potential utility as a local drug delivery system for the treatment of chronic periodontitis. Further research is warranted to validate these results in clinical settings.

Keywords: Hyaluronic acid; Pine bark extract; Periodontitis; Local drug

Introduction

Pine bark extract is a herbal supplement rich in bioactive polyphenols such as procyanidins, catecholamines, and phenolic acids. These compounds are recognized for their antimicrobial, antioxidant, and anti-inflammatory properties, contributing to various health benefits. The preparation process involves grinding pine bark, followed by washing, soaking in hot water, and extracting the liquid while discarding the solids. Hyaluronic acid (HA), a naturally occurring glycosaminoglycan, is widely utilized in periodontal therapy due to its efficacy in controlling inflammation and enhancing wound healing (1).

Periodontitis is a chronic inflammatory condition affecting the supporting structures of teeth, leading to progressive destruction of periodontal tissues. If left untreated, it can eventually result in tooth loss (1). Traditional mechanical therapies often fail to completely eliminate the subgingival microbiota responsible for disease progression, underscoring the need for adjunctive treatments. Local drug delivery systems have emerged as promising strategies, enabling the targeted and sustained release of therapeutic agents directly at the site of infection.

Hyaluronic acid plays a vital role in tissue repair and regeneration due to its high biocompatibility, viscoelastic properties, and significant water retention capacity (2). It facilitates cellular interactions, growth factor binding, and osmotic pressure regulation, making it an ideal candidate for drug delivery systems (5,7). Pine bark extract complements HA's regenerative capabilities with its well-documented anti-inflammatory and antimicrobial properties (3,4). The combination of these two components is expected to create a synergistic effect, effectively addressing the multifaceted nature of periodontal infections.

The formulation process involves combining HA and pine bark extract to produce a gel with optimal rheological properties for local application. This gel is designed for administration into periodontal pockets, ensuring the controlled release of active components. The controlled release mechanism enhances the bioavailability of therapeutic agents, allowing them to interact effectively with periodontal tissues while minimizing systemic exposure and reducing potential side effects (6).

The combination of HA and pine bark extract offers a comprehensive approach to periodontal therapy. The synergistic action of HA's regenerative potential and the antimicrobial and anti-inflammatory effects of pine bark extract provides a novel therapeutic avenue for managing periodontitis. This innovative gel formulation shows promise as an effective local drug delivery system for improving periodontal health outcomes.The aim of this study is to evaluate the antioxidant, spreadability and antiinflammatory properties of pine bark extract as a local drug delivery agent when combined with hyaluronic acid gel, assessing its potential as an adjunct in the treatment of periodontitis.

Materials and methods:

Preparation of Hyaluronic Acid-Pine Extract Gel

The Hyaluronic acid-pine extract gel was formulated by soaking 60 grams of pine bark powder, obtained commercially, in 500 ml of 95% ethanol. The mixture was kept undisturbed at room temperature for 48 hours. Following the soaking period, the solution was filtered using Whatman filter paper to obtain a clear filtrate. A 2% hyaluronic acid gel was then infused and combined with increasing concentrations of the pine extract. Gallic acid, known for its potent antioxidant properties, was used as a positive control, while DPPH solution served as the negative control to minimize errors during incubation (Figure 1).

Antioxidant Activity Analysis

The antioxidant activity of the prepared hyaluronic acid-pine extract gel was assessed using a DPPH assay. To prepare the DPPH solution, 100 ml of ethanol was mixed with 4 mg of DPPH to achieve a concentration of 0.1 mM. Pine bark extract was added to this solution in varying concentrations (25, 50, and 100 mg). Radical scavenging activity was evaluated by measuring the absorbance at 517 nm using an ultraviolet-visible spectrophotometer. A pure DPPH solution (20 mg/ml) was also tested under identical conditions as a reference standard.

Anti- Inflammatory Property Evaluation

The anti-inflammatory activity of the gel was tested by evaluating its ability to inhibit albumin denaturation. Different concentrations of the pine extract (100, 300, and 500 mg) were added to 1.5 ml of a 2% bovine serum albumin solution prepared in 0.05 M Tris-HCl buffer, with the buffer used to adjust the pH of the final solution. The samples were incubated at room temperature for 30 minutes, followed by heating in a water bath at 75 °C for 10 minutes. After cooling to room temperature, the turbidity of the samples was measured at 660 nm using an ultraviolet-visible spectrophotometer. The percentage inhibition of albumin denaturation was calculated.

Spreadability Analysis

The spreadability of the gel was evaluated by placing 2 g of the prepared gel on a glass slide (75 × 25 mm) using a pipette. A second glass slide of approximately 1 g thickness was used to

spread the gel evenly. The time required for the gel to spread over the microbial plate was recorded, and the spread diameter was measured after 1 minute for each plate.

This systematic approach ensured accurate preparation and assessment of the hyaluronic acid-pine extract gel for its antioxidant, anti-inflammatory, and spreadability properties.

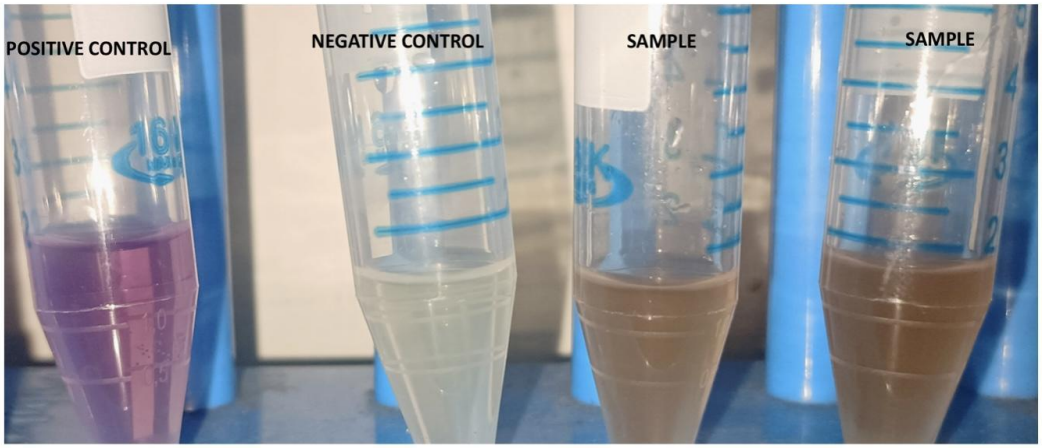

Figure-1: Sample contains Ethanolic extract of pine in combination with 2% hyaluronic acid gel,Positive control -Gallic acid, Negative control-DPPH solution

Results

The results demonstrated a direct relationship between the concentration of the pine extract and its antioxidant and anti-inflammatory activities, with both properties showing significant enhancement as the concentration increased. The data collected from the assays were tabulated, and corresponding graphs were plotted to visualize the trends.The antioxidant activity, assessed using the DPPH assay, revealed a non-linear increase with higher concentrations of the pine extract. At a concentration of 25 µg, the radical scavenging activity was measured at 30%. This value increased to 65% at 50 µg and further reached 84% at 100 µg. However, the control sample (gallic acid) exhibited 100% antioxidant activity, establishing it as the benchmark for comparison (Figure 2).

Similarly, the anti-inflammatory activity, evaluated through the inhibition of albumin denaturation, showed a progressive increase with higher concentrations of the pine extract. At 100 µL, the anti-inflammatory activity was 40%, which rose to 58% at 300 µL and peaked at 80% at 500 µL. The control sample (gallic acid), however, achieved 100% anti-inflammatory activity, highlighting its superior efficacy (Figure 3).The spreadability of the prepared gel was also assessed, with the results indicating effective application properties. The spreadability diameter of the gel was measured as 34 mm on *E. coli* microbial plates and 32 mm on *S. aureus* plates, suggesting good dispersion and even application on the microbial surfaces (Figure 4).These findings highlight that while the pine extract does not match the performance of gallic acid as a control, its increasing concentrations significantly improve both antioxidant and anti-inflammatory properties, demonstrating its potential utility as a therapeutic agent.

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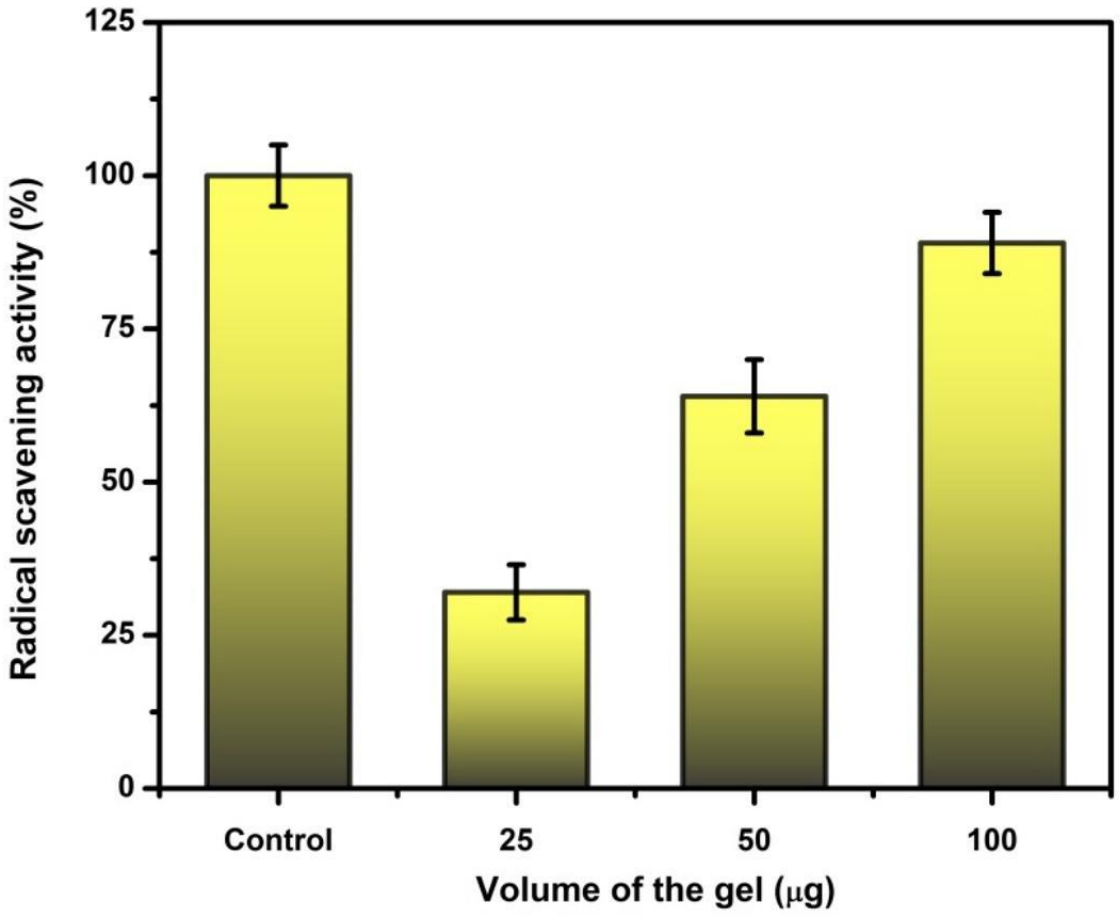

Figure-2 Graph depicting the antioxidant activity of ethanolic extract of pine- HA in comparison with the control (Gallic Acid).

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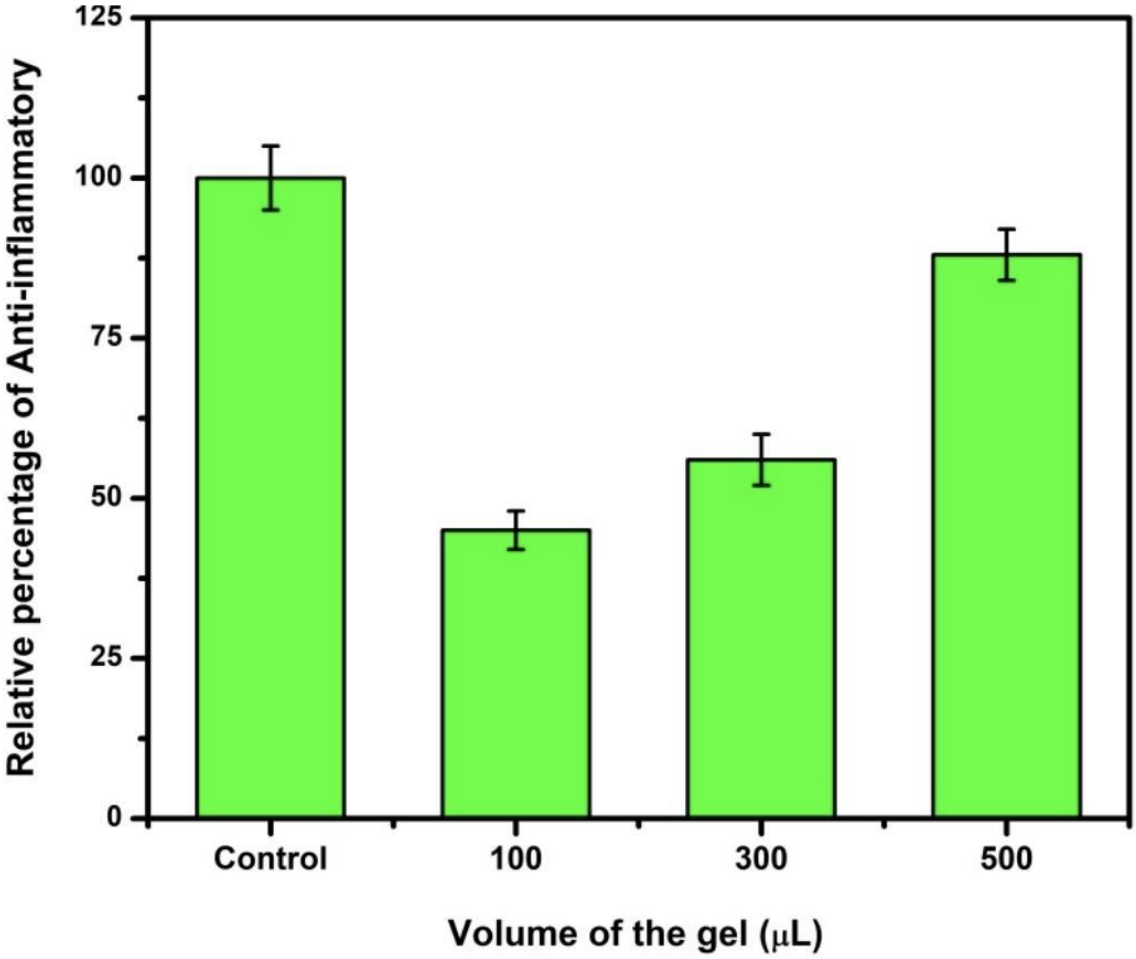

Figure-3: **Graph depicting the anti-inflammatory activity of ethanolic extract of pine and HA at 100, 300 and 500 µL when compared with the control (Gallic acid).**

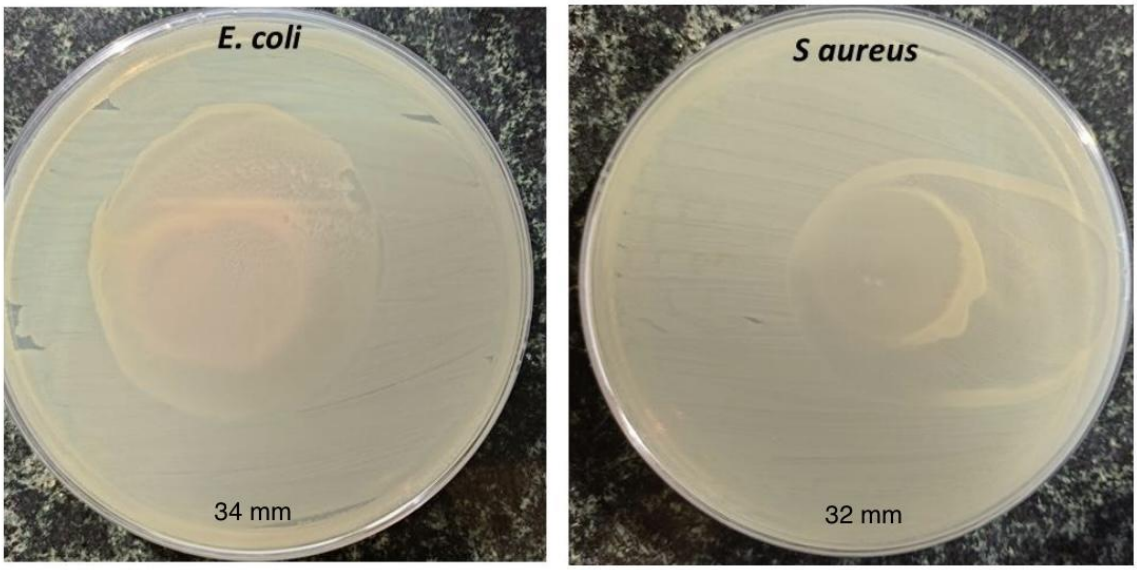

Figure.4 : Figure depicting the spreadability of the prepared extract in two different microbial plates

Discussion

The results of this study reinforce the potential of pine bark extract combined with hyaluronic acid (HA) as an innovative therapeutic agent in periodontal therapy, leveraging their individual and synergistic properties. Pine bark extract is well-documented for its significant wound-healing, anti-inflammatory, antimutagenic, and antioxidant capabilities. Similarly, HA, with its unique physicochemical and biological attributes, including hygroscopicity, bacteriostatic action, high biocompatibility, and anti-inflammatory properties, plays a crucial role in tissue repair and regeneration. Together, these components address critical aspects of periodontal treatment, as evidenced by the findings of this study.

The antioxidant activity observed in the study showed a clear concentration-dependent effect of the pine extract. At 100 µg, the radical scavenging activity reached 84%, indicating a robust antioxidant response. However, the control sample, gallic acid, demonstrated 100% activity, highlighting its superior efficacy as a standard antioxidant agent. These results are consistent with the known high antioxidant potential of pine bark extract due to its polyphenolic compounds like pycnogenol, which neutralize free radicals and protect tissues from oxidative stress. Antioxidant activity is vital in periodontal therapy as oxidative stress is a key contributor to tissue damage and inflammation.

In terms of anti-inflammatory activity, the hyaluronic acid-pine extract gel showed significant inhibition of albumin denaturation, with 80% activity observed at the highest concentration (500 µL). Although this was slightly lower than the 100% activity exhibited by the control, gallic acid,

it underscores the anti-inflammatory potential of the gel. HA is known for its role in mitigating inflammation by controlling the damage during the inflammatory process and enhancing tissue healing (7). The anti-inflammatory properties of pine bark extract, attributed to its bioactive compounds, further complement HA by reducing inflammatory markers and supporting tissue repair.

The spreadability of the gel was found to be effective, with diameters of 34 mm and 32 mm on *E. coli* and *S. aureus*plates, respectively. This result highlights the practicality of the gel for local application, ensuring even distribution and sustained release of active components. The ability of HA to form a lubricating boundary layer (11) enhances its applicability in periodontal pockets, promoting better contact with the targeted tissues.

The literature supports the versatile applications of HA in fields such as ophthalmology, orthopedics, and dentistry, with its role in periodontal therapy being well-established (13,14). The findings align with previous studies that demonstrate the efficacy of pine bark derivatives, including pycnogenol, in preventing alveolar bone resorption and combating microbial infections in dentistry (16). The antibacterial, antifungal, and osteoinductive properties of HA and the complementary actions of pine bark extract suggest their combined use as a promising therapeutic strategy in managing periodontitis.

Conclusion:

The hyaluronic acid-pine extract gel shows substantial antioxidant, anti-inflammatory, and spreadability properties, making it a viable candidate for local drug delivery in periodontal therapy. While the gel's efficacy was slightly lower than the control, its multifaceted benefits highlight its potential as an adjunct in periodontal care. Further studies, including clinical trials, are recommended to validate these findings and optimize the formulation for therapeutic use.

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