

Gelatin-Alginate Biopolymeric Matrix Enriched with *Punica granatum* and *Moringa oleifera*: In vitro Biological Evaluation for the Management of Periodontal Disease

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ABSTRACT

Background: Periodontal disease is a chronic inflammatory disorder characterized by microbial dysbiosis, oxidative stress, and exaggerated host immune response, leading to progressive periodontal tissue destruction. Natural phytoconstituents with antioxidant and anti-inflammatory properties have gained attention as potential adjuncts in periodontal therapy. To develop and biologically evaluate a gelatin-alginate biopolymeric matrix enriched with *Punica granatum* and *Moringa oleifera* for its *in vitro* antioxidant and anti-inflammatory potential relevant to periodontal disease management. **Materials and Methods:** Aqueous extracts of *Punica granatum* peel and *Moringa oleifera* leaves were incorporated into a 2% gelatin–2% sodium alginate matrix. Antioxidant activity was assessed using DPPH radical scavenging, Ferric Reducing Antioxidant Power (FRAP), and hydrogen peroxide scavenging assays. Anti-inflammatory potential was evaluated through membrane stabilization, Bovine Serum Albumin (BSA) denaturation, and egg albumin denaturation assays at concentrations ranging from 10–50 μ L. Statistical analysis was performed using one-way ANOVA and independent *t*-test, with $p < 0.05$ considered significant. **Results:** The formulation demonstrated significant concentration-dependent antioxidant and anti-inflammatory activity in all assays ($p < 0.001$). DPPH inhibition increased from 29.11% to 69.79%, FRAP values from 0.29 to 0.78 mM Fe^{2+} equivalents, and H_2O_2 scavenging from 31.25% to 71.49%. Membrane stabilization and protein denaturation assays also showed progressive inhibition, reaching up to 82.49%. No statistically significant difference was observed between the formulation and standard controls ($p > 0.05$). **Conclusion:** The phytochemical-enriched gelatin-alginate matrix exhibited potent *in vitro* antioxidant and anti-inflammatory activity comparable to standard agents, suggesting its potential as a natural adjunct in periodontal therapy. Further *in vivo* and clinical studies are warranted.

Keywords: Disease, Periodontitis, Phytotherapy, Polymers.

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Received: 06-04-2026;

Revised: 29-05-2026;

Accepted: 17-07-2026.

INTRODUCTION

Periodontal disease is a chronic inflammatory condition affecting the supporting structures of the teeth, including the gingiva, periodontal ligament, cementum, and alveolar bone. It is initiated by the accumulation of a pathogenic biofilm at the tooth-gingival interface, leading to progressive connective tissue degradation and alveolar bone resorption. If left untreated, periodontal disease ultimately results in tooth mobility and eventual tooth loss, thereby significantly compromising oral function and quality of life (Kinane, 2001).

The etiology of periodontal disease is multifactorial and centers on a dysregulated host-microbial interaction. Although specific periodontal pathogens play a pivotal role in disease initiation, tissue destruction is largely mediated by the host immune-inflammatory response. The persistent bacterial challenge triggers the release of pro-inflammatory cytokines, matrix metalloproteinases, and Reactive Oxygen Species (ROS), leading to oxidative stress and breakdown of periodontal connective tissues. Thus, contemporary understanding recognizes periodontal disease as not merely an infectious process but an inflammatory disease driven by host susceptibility and immune dysregulation (Cekici *et al.*, 2014).

Conventional management primarily involves mechanical debridement through scaling and root planing to disrupt and remove subgingival biofilm (Bhansali, 2014). Adjunctive therapies, including systemic or local antimicrobials, have been employed to enhance clinical outcomes. However, limitations such as antibiotic resistance, adverse effects, and incomplete resolution of inflammation have prompted exploration of



DOI: 10.5530/pres.20260049

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alternative therapeutic strategies that can modulate host response while exerting antimicrobial effects (Jorgensen *et al.*, 2005).

Phytotherapy has emerged as a promising adjunct in periodontal management due to its multifaceted biological activities, including antioxidant, anti-inflammatory, and antimicrobial properties. Natural bioactive compounds derived from medicinal plants offer a safer and biologically harmonious approach to controlling periodontal inflammation (Dharini *et al.*, 2024; Rukmani *et al.*, 2024; Varghese *et al.*, 2024; Srinivasan *et al.*, 2025). *Punica granatum* is rich in polyphenols and ellagitannins, which exhibit potent antioxidant and anti-inflammatory effects (Dathan *et al.*, 2024), while *Moringa oleifera* contains flavonoids and phenolic acids known for their antimicrobial and wound-healing potential (Al-Ghanayem *et al.*, 2022). These phytoconstituents may help attenuate oxidative stress and inflammatory cascades implicated in periodontal tissue destruction.

To enhance localized delivery and therapeutic efficacy, incorporation of herbal extracts into biocompatible polymeric matrices has gained attention. Gelatin, a collagen-derived biopolymer, mimics extracellular matrix components and supports cellular adhesion (Chattopadhyay *et al.*, 2014), whereas alginate provides structural stability and controlled release characteristics (Shilpa *et al.*, 2003). The synergistic integration of these polymers may facilitate sustained release of phytochemicals within the periodontal environment, thereby enhancing therapeutic outcomes.

In this context, the present study aimed to develop and biologically evaluate a gelatin-alginate biopolymeric matrix enriched with *Punica granatum* and *Moringa oleifera* for potential application in periodontal disease management.

MATERIALS AND METHODS

The study protocol was approved by the Institutional Review Board of Saveetha Dental College and Hospitals, Chennai, India (SRB/SDC/PERIO-2302/25/2961).

Preparation of Herbal Extract

2 g each of powdered *Punica granatum* peel and *Moringa oleifera* leaves were suspended separately in 100 mL of distilled water. The mixtures were heated at 50-60°C for 30 min under continuous stirring to facilitate extraction of bioactive phytoconstituents. The extracts were filtered through Whatman No. 1 filter paper and concentrated to approximately 5 mL using gentle evaporation. The extracts were stored at 4°C until further use.

Preparation of Gelatin-Alginate Biopolymeric Matrix

Gelatin (2% w/v) was dissolved in distilled water at 40-45°C under constant stirring. Sodium alginate (2% w/v) was prepared separately and mixed with the gelatin solution to obtain a homogeneous polymeric blend. The concentrated herbal extracts

were gradually incorporated into the polymer mixture under continuous stirring to ensure uniform distribution. The resulting phytochemical-loaded gelatin-alginate matrix was allowed to stabilize at room temperature prior to *in vitro* evaluation.

Antioxidant Activity 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

Aliquots of the formulation (10-50 µL) were mixed with 1 mL of 0.1 mM DPPH solution prepared in methanol and 450 µL of 50 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) buffer (pH 7.4). The reaction mixture was incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a UV-visible spectrophotometer. Butylated Hydroxytoluene (BHT) was used as the standard reference antioxidant.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-Tripyridyl-s-Triazine (TPTZ) prepared in 40 mM Hydrochloric Acid (HCl), and 20 mM ferric chloride hexahydrate (FeCl₃·6H₂O) in the ratio of 10:1:1. To 3.6 mL of freshly prepared FRAP reagent, 80 µL of the sample was added and incubated at 37°C for 10 min. The absorbance was measured at 593 nm using a UV-visible spectrophotometer. Ferrous sulfate heptahydrate (FeSO₄·7H₂O) was used to construct the standard calibration curve, and antioxidant activity was expressed as Ferrous Ion (Fe²⁺) equivalents.

Hydrogen Peroxide (H₂O₂) Scavenging Assay

Each reaction mixture (1 mL) contained 28 mM 2-deoxy-2-ribose, 200 µM ferric chloride, Ethylenediaminetetraacetic Acid (EDTA), ascorbic acid, and varying concentrations of the biocomposite. The mixture was incubated at 37°C for 1 hr, and the absorbance was recorded at 532 nm using a UV-visible spectrophotometer.

Anti-Inflammatory Activity

Membrane Stabilization Assay

Human Red Blood Cells (RBCs) were isolated from healthy volunteers following informed consent. A 10% v/v RBC suspension was prepared in Phosphate-Buffered Saline (PBS). One milliliter of the RBC suspension was mixed with varying volumes (10-50 µL) of the formulation and incubated at 37°C for 30 min under hypotonic conditions. After centrifugation at 1,000 rpm for 10 min, the absorbance of the supernatant was measured at 540 nm using a UV-visible spectrophotometer. Diclofenac sodium served as the reference standard.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

Protein Denaturation (Bovine Serum Albumin [BSA]) Assay

To 2 mL of 1% BSA solution (pH 6.8), varying volumes (10-50 μ L) of the formulation were added. The mixtures were incubated at 37°C for 20 min and subsequently cooled to room temperature. The absorbance was measured at 660 nm using a UV-visible spectrophotometer. Diclofenac sodium served as the standard reference drug.

$$\text{Inhibition (\%)} = (\text{Absorbance of control} - \text{Absorbance of sample} / \text{Absorbance of control}) \times 100$$

Egg Albumin Denaturation Assay

Fresh egg albumin (0.2 mL) was added to 2.8 mL of PBS and treated with various concentrations (10-50 μ g/mL) of the biocomposite. The samples were subjected to identical incubation and heating conditions as described for the BSA assay. The percentage inhibition of protein denaturation was calculated using the same formula.

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean $\pm$ Standard Deviation (SD). Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS) software (Version 23.0; IBM Corp., Armonk, NY, USA). One-way Analysis of Variance (ANOVA) was employed to evaluate differences among various concentrations within each experimental group. An independent samples *t*-test was used to compare the antioxidant and anti-inflammatory activities of the gelatin-alginate biopolymeric formulation with the respective standard controls at corresponding concentrations. A *p*-value of < 0.05 was considered statistically significant.

RESULTS

DPPH Radical Scavenging Assay

The formulation demonstrated a clear concentration-dependent increase in DPPH radical scavenging activity (Figure 1), rising from $29.11 \pm 3.16\%$ at 10 μ L to $69.79 \pm 3.92\%$ at 50 μ L. One-way ANOVA revealed a statistically significant difference across concentrations ($F = 58.42$, $p < 0.001$), indicating strong dose responsiveness (Table 1). Although the standard exhibited marginally higher inhibition at all concentrations, independent *t*-test analysis showed no statistically significant difference between the test formulation and standard ($p > 0.05$), suggesting comparable free radical scavenging potential (Table 2).

FRAP Assay

A progressive enhancement in ferric reducing capacity was observed with increasing concentration (Figure 2), from 0.29 ± 0.02 to 0.78 ± 0.03 mM Fe^{2+} equivalents. The variation across concentrations was highly significant ($F = 112.76$, $p < 0.001$), reflecting robust reducing power (Table 1). Despite slightly higher values in the standard group, intergroup comparison revealed no significant difference ($p > 0.05$), indicating similar antioxidant efficacy (Table 2).

H_2O_2 Scavenging Assay

The biopolymeric matrix exhibited dose-dependent hydrogen peroxide scavenging activity (Figure 3), increasing from $31.25 \pm 4.23\%$ to $71.49 \pm 3.99\%$. Statistical analysis confirmed significant variation across concentrations ($F = 49.85$, $p < 0.001$) (Table 1). While the standard showed marginally greater inhibition, the difference between groups was not statistically significant at any

Table 1: Antioxidant and anti-inflammatory activities of the gelatin-alginate matrix enriched with *Punica granatum* and *Moringa oleifera* at different concentrations.

Assay	10 μ L (Mean $\pm$ SD)	20 μ L (Mean $\pm$ SD)	30 μ L (Mean $\pm$ SD)	40 μ L (Mean $\pm$ SD)	50 μ L (Mean $\pm$ SD)	F-statistic	<i>p</i> -value ^a
DPPH (% inhibition)	29.11 ± 3.16	33.25 ± 2.89	46.17 ± 2.54	58.15 ± 3.48	69.79 ± 3.92	58.42	<0.001*
FRAP (mM Fe^{2+} equivalents)	0.29 ± 0.02	0.37 ± 0.03	0.51 ± 0.03	0.63 ± 0.03	0.78 ± 0.03	112.76	<0.001*
H_2O_2 Scavenging (% inhibition)	31.25 ± 4.23	42.45 ± 3.19	52.27 ± 3.74	63.26 ± 3.86	71.49 ± 3.99	49.85	<0.001*
Membrane Stabilization (% hemolysis inhibition)	29.79 ± 3.56	39.52 ± 2.99	52.76 ± 3.19	67.52 ± 3.09	76.27 ± 2.89	64.31	<0.001*
BSA (% inhibition)	32.84 ± 3.75	45.15 ± 3.33	64.45 ± 3.65	73.64 ± 2.72	82.49 ± 2.87	72.94	<0.001*
Egg Albumin Denaturation (% inhibition)	37.72 ± 3.15	46.24 ± 3.73	54.35 ± 3.95	66.24 ± 2.92	74.46 ± 2.77	46.18	<0.001*

^aOne-way ANOVA; **p*-value < 0.05 (Statistically Significant).

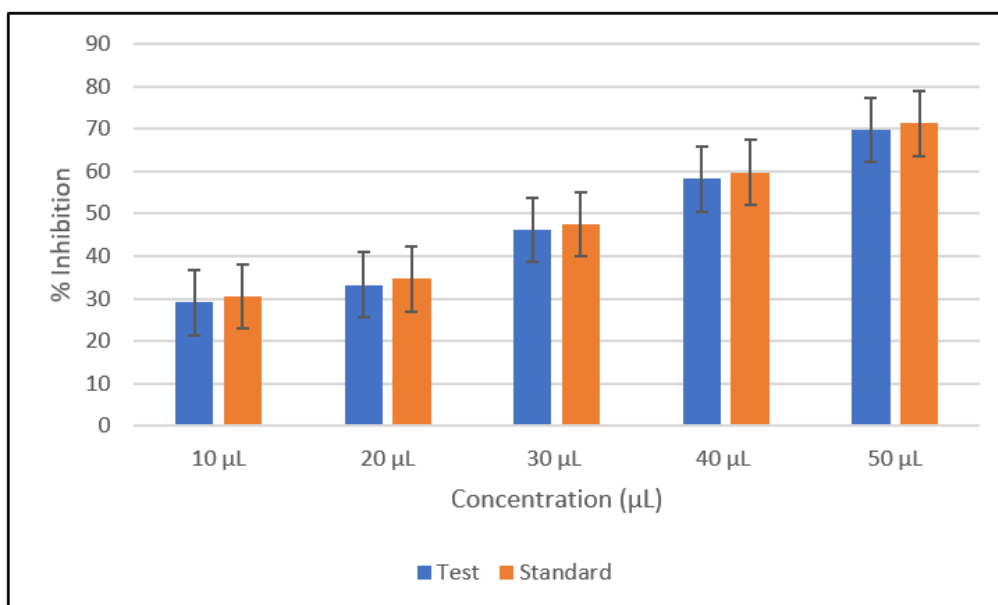


Figure 1: Concentration-dependent DPPH radical scavenging activity of the test formulation compared with standard.

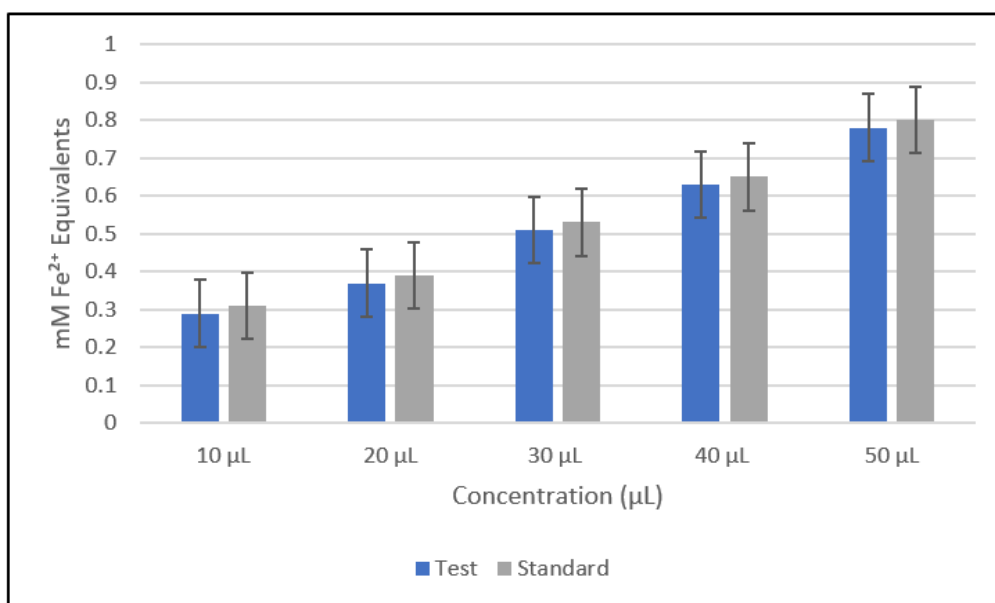


Figure 2: Ferric reducing antioxidant capacity of the test formulation versus standard across concentrations.

concentration ($p > 0.05$), demonstrating comparable peroxide neutralizing ability (Table 2).

Membrane Stabilization Assay

Inhibition of hemolysis increased steadily from $29.79 \pm 3.56\%$ at $10 \mu\text{L}$ to $76.27 \pm 2.89\%$ at $50 \mu\text{L}$ (Figure 4). The concentration-dependent effect was statistically significant ($F = 64.31, p < 0.001$) (Table 1). Although diclofenac sodium exhibited slightly higher stabilization, independent t-test analysis showed no significant intergroup difference ($p > 0.05$), indicating similar membrane protective activity (Table 2).

BSA Denaturation Assay

The formulation effectively inhibited protein denaturation in a dose-dependent manner (Figure 5), with inhibition increasing from $32.84 \pm 3.75\%$ to $82.49 \pm 2.87\%$. ANOVA demonstrated significant differences across concentrations ($F = 72.94, p < 0.001$) (Table 1). Despite marginally superior inhibition by the standard, intergroup comparisons revealed no statistically significant difference ($p > 0.05$), suggesting comparable anti-inflammatory potential (Table 2).

Egg Albumin Denaturation Assay

A concentration-dependent inhibition of egg albumin denaturation was observed (Figure 6), ranging from $37.72 \pm 3.15\%$ to $74.46 \pm 2.77\%$. The variation across concentrations was statistically significant ($F = 46.18$, $p < 0.001$) (Table 1). Although the standard showed slightly higher inhibition values, the differences were not statistically significant ($p > 0.05$), indicating similar protein stabilization capacity (Table 2).

DISCUSSION

Periodontal disease is a chronic inflammatory disorder characterized by persistent microbial challenge and an exaggerated host immune response, resulting in excessive production of ROS and inflammatory mediators. Oxidative stress plays a central role in connective tissue degradation and alveolar bone resorption, thereby accelerating disease progression. Contemporary therapeutic strategies therefore emphasize not only microbial control but also host modulation. In this regard, phytotherapy has gained increasing attention as a biologically harmonious approach due to its antioxidant, anti-inflammatory, and tissue-protective properties (Thaha *et al.*, 2025; Krishna *et al.*, 2024; Manohar *et al.*, 2025).

Among medicinal plants, *Punica granatum* and *Moringa oleifera* have been extensively documented for their rich phytochemical profiles. Pomegranate peel extracts are abundant in polyphenols, ellagitannins, flavonoids, and tannins, which exhibit strong free radical scavenging, metal-chelating, and lipid peroxidation inhibitory activities (Sihag *et al.*, 2022). Previous studies have reported remarkably low half maximal inhibitory concentration (IC_{50}) values in DPPH, nitric oxide, and hydroxyl

radical assays, highlighting their potent antioxidant capacity (Ismail *et al.*, 2012; Silla *et al.*, 2025). Similarly, moringa leaves are rich in flavonoids such as quercetin and kaempferol, along with phenolic acids, which contribute to significant DPPH, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and FRAP activity, nitric oxide inhibition, membrane stabilization, and prevention of protein denaturation (Saleem *et al.*, 2020). Experimental evidence has also demonstrated anti-arthritic and anti-inflammatory effects of moringa through modulation of oxidative stress and inflammatory mediators (Xu *et al.*, 2019; Coz-Bolaños *et al.*, 2018). Collectively, these findings support the rationale for combining these botanicals to enhance therapeutic efficacy.

Recent formulation-based investigations suggest that combining pomegranate peel and moringa leaf extracts may produce synergistic enhancement of total phenolic content and antioxidant potential (Meghwar *et al.*, 2024). Building upon this scientific foundation, the present study developed a gelatin-alginate biopolymeric matrix enriched with *Punica granatum* and *Moringa oleifera* and evaluated its *in vitro* biological activity with relevance to periodontal disease management.

The antioxidant assays demonstrated a clear and statistically significant concentration-dependent response. In the DPPH assay, the formulation exhibited progressive radical scavenging activity, indicating effective hydrogen-donating ability. The FRAP assay further confirmed strong electron-donating capacity, reflecting its potential to reduce oxidized intermediates within inflamed periodontal tissues. Similarly, hydrogen peroxide scavenging activity increased significantly with concentration, suggesting effective neutralization of peroxide species that could

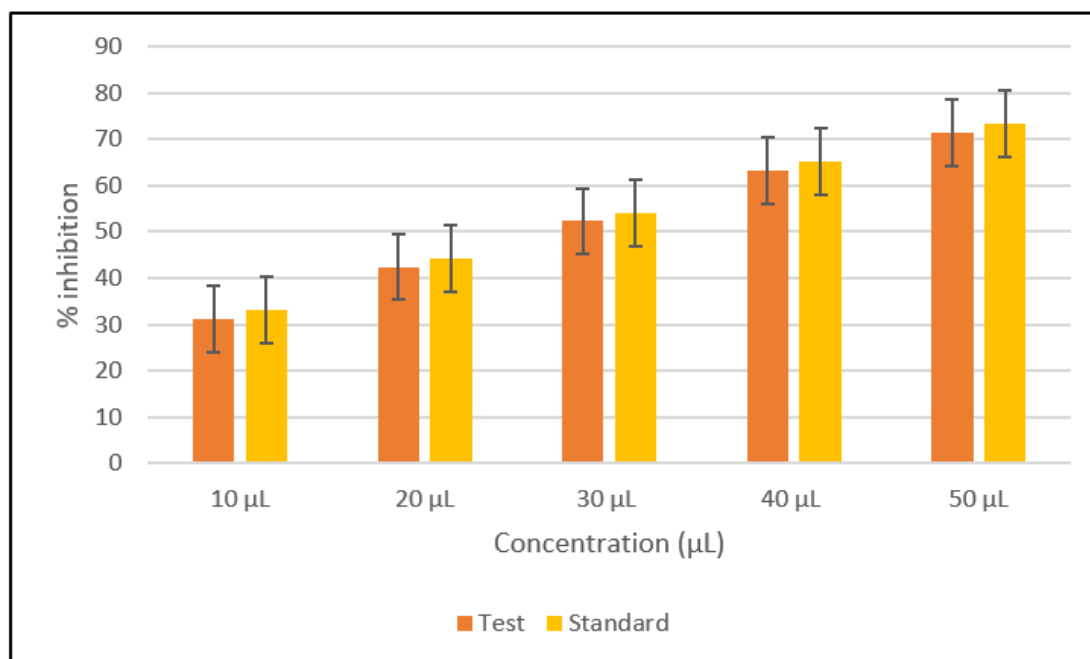


Figure 3: Hydrogen peroxide scavenging activity of the test formulation compared with standard.

Table 2: Comparison of the formulation (Test) with standard controls.

Assay	10 μ L (Mean $\pm$ SD)	20 μ L (Mean $\pm$ SD)	30 μ L (Mean $\pm$ SD)	40 μ L (Mean $\pm$ SD)	50 μ L (Mean $\pm$ SD)
DPPH – Test	29.11 $\pm$ 3.16	33.25 $\pm$ 2.89	46.17 $\pm$ 2.54	58.15 $\pm$ 3.48	69.79 $\pm$ 3.92
DPPH – Standard	30.48 $\pm$ 3.42	34.62 $\pm$ 3.11	47.54 $\pm$ 2.77	59.73 $\pm$ 3.65	71.26 $\pm$ 4.08
DPPH – <i>t</i> -value	0.74	0.81	0.89	0.92	0.85
DPPH – <i>p</i> -value ^b	0.472	0.436	0.391	0.378	0.409
FRAP – Test	0.29 $\pm$ 0.02	0.37 $\pm$ 0.03	0.51 $\pm$ 0.03	0.63 $\pm$ 0.03	0.78 $\pm$ 0.03
FRAP – Standard	0.31 $\pm$ 0.03	0.39 $\pm$ 0.03	0.53 $\pm$ 0.04	0.65 $\pm$ 0.03	0.80 $\pm$ 0.04
FRAP – <i>t</i> -value	1.12	1.05	0.98	1.03	0.96
FRAP – <i>p</i> -value ^b	0.286	0.314	0.348	0.322	0.356
H ₂ O ₂ Scavenging – Test	31.25 $\pm$ 4.23	42.45 $\pm$ 3.19	52.27 $\pm$ 3.74	63.26 $\pm$ 3.86	71.49 $\pm$ 3.99
H ₂ O ₂ Scavenging – Standard	33.02 $\pm$ 4.48	44.18 $\pm$ 3.41	54.11 $\pm$ 3.89	65.08 $\pm$ 4.02	73.36 $\pm$ 4.11
H ₂ O ₂ Scavenging – <i>t</i> -value	0.73	0.82	0.85	0.88	0.79
H ₂ O ₂ Scavenging – <i>p</i> -value ^b	0.476	0.428	0.407	0.392	0.442
Membrane Stabilization – Test	29.79 $\pm$ 3.56	39.52 $\pm$ 2.99	52.76 $\pm$ 3.19	67.52 $\pm$ 3.09	76.27 $\pm$ 2.89
Membrane Stabilization – Standard	31.44 $\pm$ 3.74	41.21 $\pm$ 3.16	54.38 $\pm$ 3.31	69.14 $\pm$ 3.22	78.03 $\pm$ 3.05
Membrane Stabilization – <i>t</i> -value	0.82	0.91	0.86	0.94	0.89
Membrane Stabilization – <i>p</i> -value ^b	0.427	0.383	0.401	0.371	0.394
BSA – Test	32.84 $\pm$ 3.75	45.15 $\pm$ 3.33	64.45 $\pm$ 3.65	73.64 $\pm$ 2.72	82.49 $\pm$ 2.87
BSA – Standard	34.52 $\pm$ 3.98	47.02 $\pm$ 3.51	66.18 $\pm$ 3.84	75.32 $\pm$ 2.89	84.21 $\pm$ 3.03
BSA – <i>t</i> -value	0.79	0.88	0.83	0.91	0.86
BSA – <i>p</i> -value ^b	0.441	0.398	0.419	0.382	0.403
Egg Albumin Denaturation – Test	37.72 $\pm$ 3.15	46.24 $\pm$ 3.73	54.35 $\pm$ 3.95	66.24 $\pm$ 2.92	74.46 $\pm$ 2.77
Egg Albumin Denaturation – Standard	39.31 $\pm$ 3.32	48.06 $\pm$ 3.89	56.08 $\pm$ 4.11	68.01 $\pm$ 3.08	76.22 $\pm$ 2.94
Egg Albumin Denaturation – <i>t</i> -value	0.87	0.81	0.85	0.93	0.90
Egg Albumin Denaturation – <i>p</i> -value ^b	0.399	0.432	0.406	0.376	0.389

^bIndependent *t*-test; *p*-value < 0.05 (Statistically Significant).

otherwise generate highly reactive hydroxyl radicals. Importantly, although standard antioxidants showed marginally higher values, independent *t*-test analysis revealed no statistically significant difference between the formulation and standard controls, indicating comparable antioxidant efficacy.

The anti-inflammatory assays further substantiated the biological potential of the developed matrix. Membrane stabilization activity increased significantly with concentration, reflecting protection against hypotonicity-induced hemolysis. Since erythrocyte membrane stabilization is considered analogous to lysosomal membrane stabilization during inflammation, this finding suggests potential inhibition of inflammatory mediator release.

Additionally, the formulation significantly inhibited protein denaturation in both BSA and egg albumin models. Protein denaturation is a well-recognized mechanism contributing to inflammatory processes; therefore, its inhibition highlights the formulation's capacity to modulate inflammatory pathways. As observed in antioxidant assays, the activity was comparable to diclofenac sodium, with no statistically significant intergroup differences.

The incorporation of herbal extracts into a gelatin–alginate matrix may have enhanced their functional performance. Gelatin, being collagen-derived, mimics extracellular matrix components and supports tissue compatibility, while alginate provides structural

stability and potential controlled release characteristics (Estevez *et al.*, 2024). This polymeric integration may facilitate sustained retention of phytoconstituents within the periodontal pocket, thereby optimizing localized antioxidant and anti-inflammatory effects. Such a delivery system aligns well with the concept of adjunctive host modulation therapy in periodontal management.

Overall, the findings of this study are consistent with existing literature highlighting the potent antioxidant and anti-inflammatory properties of *Punica granatum* and *Moringa oleifera*. The developed biopolymeric formulation demonstrated significant dose-dependent biological activity and efficacy comparable to standard agents, supporting its potential as a natural adjunct in periodontal therapy.

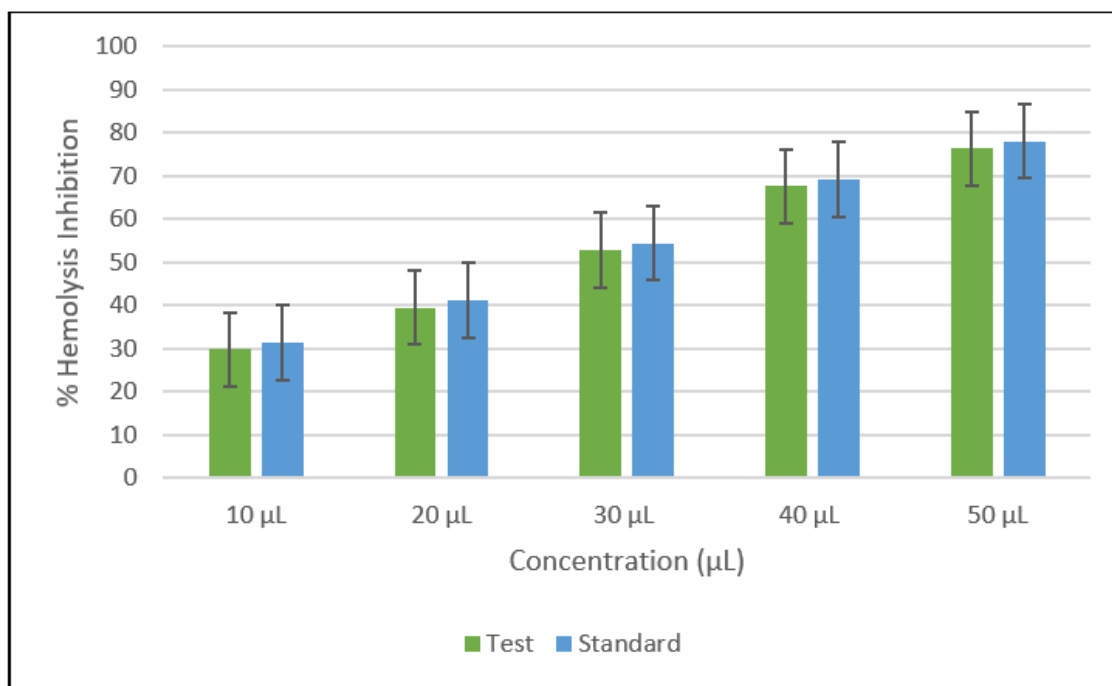


Figure 4: Percentage inhibition of hemolysis by the test formulation compared with standard.

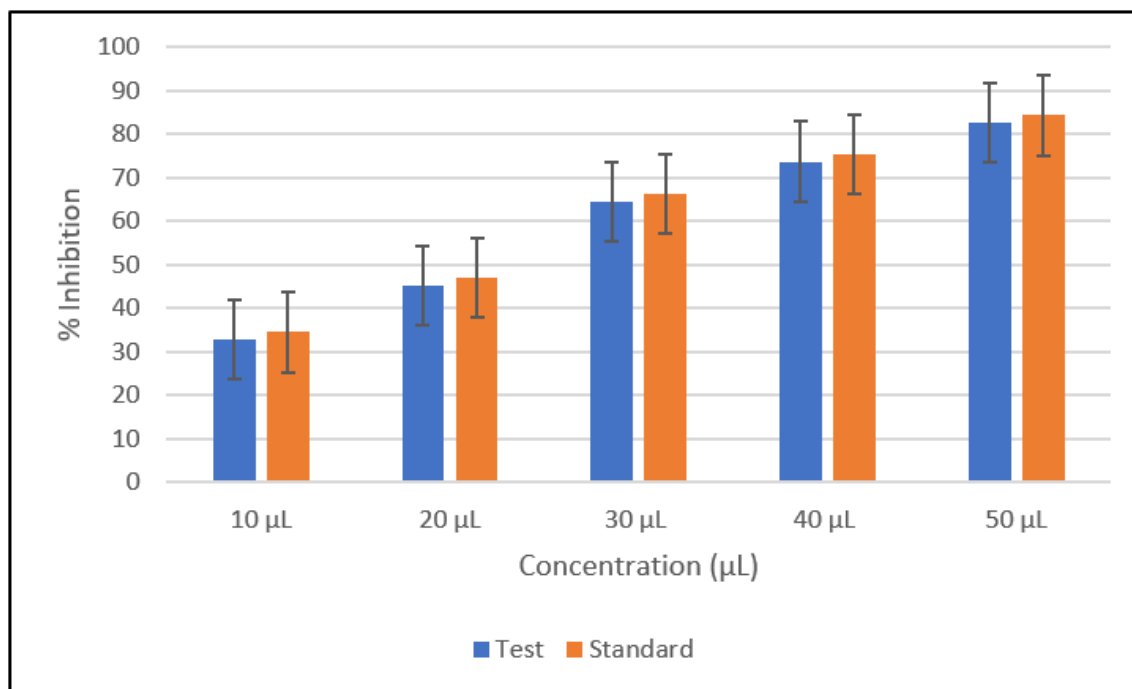


Figure 5: Percentage inhibition of protein denaturation (BSA) by the test formulation compared with standard.

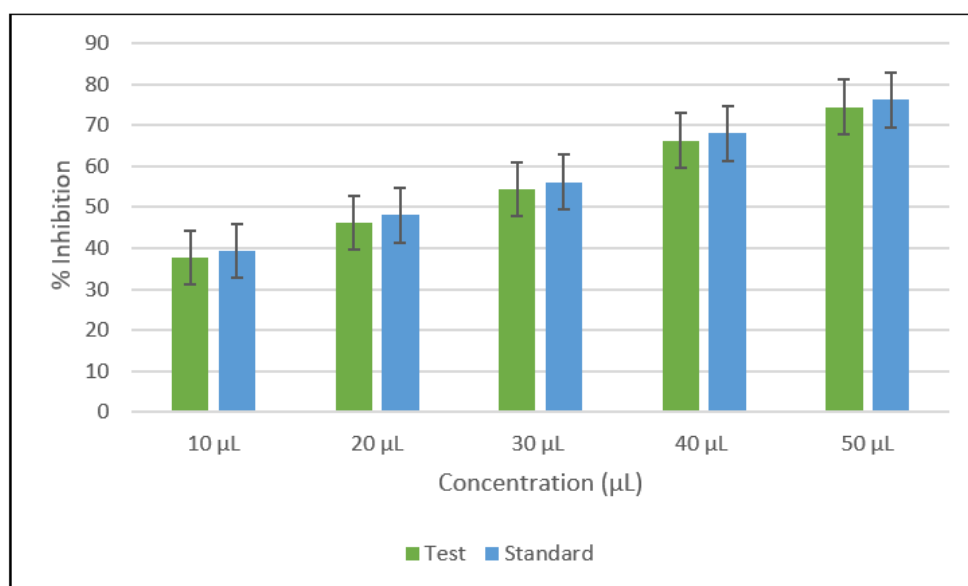


Figure 6: Percentage inhibition of egg albumin denaturation by the test formulation compared with standard.

However, certain limitations should be acknowledged. The evaluation was confined to *in vitro* models, which may not fully replicate the complex host–microbial interactions and dynamic inflammatory environment present in periodontal tissues. Antimicrobial activity against key periodontal pathogens was not assessed, and detailed phytochemical characterization, cytocompatibility testing, and release kinetics studies were not performed. Future investigations should therefore include antimicrobial evaluation, biocompatibility studies using oral cell lines, controlled release profiling, and *in vivo* periodontal models to validate therapeutic efficacy. Ultimately, well-designed clinical trials will be essential to establish the safety, effectiveness, and translational potential of this phytochemical-enriched biopolymeric matrix in periodontal disease management.

CONCLUSION

The developed gelatin-alginate matrix enriched with *Punica granatum* and *Moringa oleifera* exhibited significant *in vitro* antioxidant and anti-inflammatory activity, comparable to standard agents. These findings suggest its potential as a natural adjunct in periodontal therapy. Further *in vivo* and clinical validation is warranted.

ACKNOWLEDGEMENT

The authors would like to acknowledge Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), for providing the necessary infrastructure and facilities to carry out this research.

ABBREVIATIONS

ANOVA: Analysis of Variance; **BHT:** Butylated Hydroxytoluene; **BSA:** Bovine Serum Albumin; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **EDTA:** Ethylenediaminetetraacetic acid; **FRAP:** Ferric Reducing Antioxidant Power; **HCl:** Hydrochloric Acid; **PBS:** Phosphate-Buffered Saline; **RBC:** Red Blood Cell; **ROS:** Reactive Oxygen Species; **SD:** Standard Deviation; **TPTZ:** 2,4,6-tripyridyl-s-triazine; **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); **IC₅₀:** Half maximal inhibitory concentration.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

VB: Conceptualization, Methodology, Data curation, Writing – Original draft. AR: Supervision, Validation, Review & Editing, Project administration.

SUMMARY

Periodontal disease is a chronic inflammatory condition driven by oxidative stress and immune dysregulation. This study developed a gelatin-alginate biopolymeric matrix enriched with *Punica granatum* and *Moringa oleifera* extracts to serve as a local drug delivery system. *In vitro* evaluation confirmed that the formulation possesses significant antioxidant and anti-inflammatory properties, comparable to standard agents like Diclofenac and BHT. The matrix effectively scavenged free radicals (DPPH, H₂O₂, FRAP) and inhibited protein denaturation and hemolysis. These findings suggest that this herbal-loaded

biopolymer is a promising, biocompatible adjunct for managing periodontal inflammation.

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Cite this article: Batra V, Rajasekar A. Gelatin-Alginate Biopolymeric Matrix Enriched with *Punica granatum* and *Moringa oleifera*: *In vitro* Biological Evaluation for the Management of Periodontal Disease. *Pharmacog Res*. 2026;18(4):1489–97.