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Original Article

Exploring the Therapeutic Potential of a Gelatin-Based Biocomposite Loaded with *Moringa oleifera* and *Camellia sinensis*: Anti-Inflammatory and Antioxidant Insights

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ABSTRACT

Introduction: Oxidative stress and chronic inflammation are central to the progression of numerous disorders. Natural bioactive compounds capable of modulating these processes are of growing interest in regenerative medicine. *Moringa oleifera* and *Camellia sinensis* are rich in flavonoids, phenolic acids, and catechins, which exhibit potent antioxidant, anti-inflammatory, and tissue-protective properties. Incorporating these extracts into a gelatin-based matrix offers a novel strategy for developing biofunctional composites with controlled release and enhanced stability. **Aim:** This study aimed to prepare and characterize a gelatin-based biocomposite loaded with *Moringa oleifera* and *Camellia sinensis* and to evaluate its *in vitro* anti-inflammatory and antioxidant potential. **Materials and Methods:** Aqueous extracts of *Moringa oleifera* and *Camellia sinensis* leaves powder were incorporated into a 2% (w/v) gelatin solution to form a stable biocomposite. Anti-inflammatory activity was assessed using Bovine Serum Albumin (BSA) and egg albumin denaturation assays, while antioxidant activity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl **(**DPPH) radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. Concentration-dependent effects were analyzed using one-way ANOVA, and comparisons with standard compounds were performed using independent *t*-tests. **Results:** The biocomposite exhibited a concentration-dependent increase in both anti-inflammatory and antioxidant activity, comparable to standard reference compounds across all assays ($p<0.05$). Inter-group analysis showed no statistically significant differences between the biocomposite and standards ($p>0.05$), indicating equivalent efficacy. **Conclusion:** The gelatin-based biocomposite containing *Moringa oleifera* and *Camellia sinensis* demonstrates potent antioxidant and anti-inflammatory activity, highlighting its potential as a natural therapeutic platform for inflammation-related applications.

Keywords: Herbal Extracts, Inflammation, Oxidative Stress, Phytotherapy.

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INTRODUCTION

Inflammation and oxidative stress are critical contributors to the onset and progression of numerous chronic disorders, including periodontal diseases, cardiovascular conditions, and metabolic dysfunctions (Bellanti *et al*., 2025). An imbalance between free radical generation and antioxidant defense leads to tissue injury, delayed wound healing, and impaired regenerative outcomes (Ukaegbu K *et al*., 2025). Hence, the search for biomaterials capable of delivering bioactive compounds with anti-inflammatory and antioxidant properties has gained significant momentum in biomedical and dental research (Dathan PC *et al*., 2024), (Dharini S *et al*., 2024), (Krishna KN *et al*., 2024), (Rukmani PA *et al*., 2024), (Varghese RM *et al*., 2024), (Manohar JH *et al*., 2025), (Srinivasan S *et al*., 2025), (Thaha M *et al*., 2025).

Gelatin, a naturally derived biopolymer obtained from collagen, is widely recognized for its biocompatibility, biodegradability, and ability to form stable matrices. Its structural flexibility and safety profile make it an excellent candidate for developing drug delivery systems and bio functional composites. Incorporating phytochemicals into a gelatin framework enhances the material's therapeutic properties while preserving its physicochemical stability (Jia X *et al*., 2024).

Moringa oleifera, commonly known as the drumstick tree, is a medicinal plant whose leaves are rich in flavonoids, phenolic acids, vitamins, and essential amino acids. These bioactive constituents

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contribute to its strong antioxidant capacity and well-documented anti-inflammatory, antimicrobial, and wound-healing effects (Zarina *et al*., 2024). *Camellia sinensis* (green tea) is another botanical of profound therapeutic interest, predominantly due to Epigallocatechin Gallate (EGCG) and other catechins that exhibit free radical scavenging, anti-inflammatory, and tissue-protective functions (Mokra D *et al*., 2022). When combined, these extracts are anticipated to act synergistically, strengthening cellular defense mechanisms and modulating inflammatory pathways.

Developing a gelatin-based bio composite infused with *Moringa oleifera* and *Camellia sinensis* presents a novel therapeutic strategy aimed at enhancing anti-inflammatory and antioxidant responses. Such a formulation holds promise for application in regenerative medicine and dental biomaterials, where controlling oxidative stress and inflammation is crucial for favorable clinical outcomes. The present *in vitro* study was designed to prepare and characterize this bio composite and to evaluate its bioactivity using validated anti-inflammatory and antioxidant assays.

MATERIALS AND METHODS

Preparation of the Biocomposite

Two grams each of *Moringa oleifera* leaf powder and *Camellia sinensis* leaf powder were accurately weighed and suspended in 100 mL of distilled water. The mixture was stirred and heated at 50-60 °C to facilitate the extraction of bioactive phytochemicals without affecting thermolabile compounds. The extract was filtered through Whatman No. 1 filter paper and concentrated to a final volume of 5 mL. Separately, a 2% (w/v) gelatin solution was prepared by dissolving gelatin in warm distilled water under continuous stirring until homogeneous. The concentrated plant extract was incorporated dropwise into the gelatin solution with constant agitation to obtain a stable gelatin-based bio composite (Haroun AA *et al*., 2010).

Anti-Inflammatory Activity

Bovine Serum Albumin (BSA) Protein Denaturation Assay

A 0.45 mL aliquot of BSA was mixed with 0.05 mL of the bio composite (10-50 µg/mL). The pH was adjusted to 6.3, followed by incubation at room temperature for 10 min and heating at 55 °C for 30 min. Diclofenac sodium served as the positive control, and Dimethyl Sulfoxide (DMSO) was used as the negative control (Rubinchik E *et al*., 2010). Absorbance was measured at 660 nm, and percentage inhibition was calculated using:

$$\% \text{ Inhibition} = [(\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control}] \times 100$$

Egg Albumin Denaturation Assay

Fresh egg albumin (0.2 mL) was mixed with 2.8 mL phosphate-buffered saline (PBS) and treated with the bio composite at concentrations of 10-50 µg/mL. Samples underwent the same incubation and heating conditions as in the BSA assay. Absorbance was recorded, and inhibition was calculated using the above formula (Rubinchik E *et al*., 2010).

Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

Different concentrations (10-50 µg/mL) of the bio composite were mixed with 20 µM DPPH solution in methanol and incubated in the dark at room temperature for 30 min. Absorbance was measured at 517 nm. Ascorbic acid (1 mg/mL) served as the standard, while methanol was used as the blank (da Rosa JS *et al*., 2024). Radical scavenging activity was calculated as:

$$\% \text{ Scavenging} = [(\text{Abs Control} - \text{Abs Sample}) / \text{Abs Control}] \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP reagent was freshly prepared by mixing acetate buffer (300 mM, pH 3.6), 2,4,6-tripyridyl-s-triazine (TPTZ, 10 mM in 40 mM HCl), and ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 20 mM) in a 10:1:1 ratio. The bio composite extract was combined with the FRAP reagent and incubated at 37 °C for 10 min. Absorbance was measured at 593 nm, indicating the formation of a ferrous-TPTZ complex (da Rosa JS *et al*., 2024).

Statistical Analysis

Data were expressed as Mean ± Standard Deviation (SD). Intra-group comparisons across increasing concentrations were performed using one-way ANOVA to assess dose-dependent effects within each group. Inter-group comparisons at each concentration were conducted using independent *t*-test to evaluate differences between test and standard samples. A p-value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 23.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The Mean ± SD values of both test and standard groups demonstrated a concentration-dependent increase across all assays. In the BSA assay, test values increased from 39.22 ± 5.45 at 10 µg/mL to 70.19 ± 5.79 at 50 µg/mL ($p = 0.002$), while standard values increased from 36.55 ± 3.78 to 68.84 ± 2.79 ($p = 0.003$). In egg albumin denaturation, test values increased from 28.63 ± 4.33 to 53.74 ± 3.79 ($p = 0.000$), and standard values from 28.62 ± 4.22 to 54.46 ± 2.44 ($p = 0.001$). For DPPH assay, test values increased from 29.34 ± 4.76 to 55.12 ± 4.76 ($p = 0.001$), and standard values from 30.48 ± 2.73 to 55.89 ± 2.62 ($p = 0.002$). In the FRAP assay, test values increased from 0.27 ± 0.05 to 0.62 ± 0.04 ($p = 0.000$), while standard values increased from 0.32 ± 0.05 to 0.64 ± 0.06 ($p = 0.001$). Inter-group comparisons at each concentration revealed no statistically significant differences between test and standard

across all assays ($p > 0.05$), indicating comparable efficacy of the test sample relative to the standard at all concentrations (Table 1).

DISCUSSION

Oxidative stress and chronic inflammation are central contributors to tissue damage and impaired healing in various pathological conditions. Natural plant-derived compounds with antioxidant and anti-inflammatory properties are increasingly recognized as effective and safer alternatives to synthetic agents. Among these, *Moringa oleifera* and *Camellia sinensis* are particularly notable due to their rich phytochemical content, including flavonoids, phenolic acids, and catechins, which confer potent free radical scavenging, anti-inflammatory, and tissue-protective activities.

The present study aimed to evaluate the therapeutic potential of a gelatin-based bio composite loaded with Moringa oleifera and Camellia sinensis extracts, focusing on their combined anti-inflammatory and antioxidant effects. To the best of our knowledge, this is the first study of its kind to investigate a dual-botanical formulation within a gelatin matrix, providing both structural stability and controlled release of bioactive compounds.

The results demonstrated a clear concentration-dependent increase in antioxidant and anti-inflammatory activity in the test bio composite, with comparable efficacy to standard reference compounds. These findings are in alignment with existing literature. An *in vitro* study has reported that aqueous and ethanolic *Moringa oleifera* extracts exhibited significant antioxidant and anti-inflammatory activity, suggesting their potential for local therapeutic applications (Ramamurthy S *et al*., 2022). Similarly another study highlighted the anti-inflammatory efficacy of *Moringa* extracts, although their antioxidant effects were moderate (Alhakmani F *et al*., 2013). Furthermore *Moringa* extracts showed concentration-dependent antioxidant and anti-inflammatory properties in both *in vitro* and animal models, including reductions in oxidative stress markers and pro-inflammatory cytokines (Adedapo AA *et al*., 2015), (Omodanisi EI *et al*., 2017), (Avilés-Gaxiola S *et al*., 2021).

Camellia sinensis has been extensively studied for its catechin-rich extracts. Literature evidence has demonstrated antioxidant, anti-inflammatory, and hepatoprotective effects of green tea leaf extracts (Thitimuta S *et al*., 2017). Furthermore, reported its analgesic and anti-inflammatory activity was demonstrated in murine models (Mota MA *et al*., 2015). Research has alsoelucidated the molecular mechanisms by which *Camellia sinensis* extracts inhibit cytokine production and modulate inflammatory signaling pathways in keratinocytes (Kim MJ *et al*., 2025). Even green tea flower extracts possess potent anti-inflammatory effects, broadening the therapeutic scope of this plant (Chen BT *et al*., 2012). Collectively, these studies support the findings of the present investigation, emphasizing that the combination of *Moringa oleifera* and *Camellia sinensis* may offer synergistic benefits.

The choice of gelatin as a biopolymer was deliberate. Gelatin provides a biocompatible, biodegradable, and hydrophilic matrix that ensures sustained release of bioactive compounds while maintaining their stability. Its gelation properties also facilitate easy formulation into hydrogels or films, which can be adapted for local delivery in periodontal therapy, wound healing, or other biomedical applications.

Strengths of this study include the use of multiple complementary assays to comprehensively evaluate antioxidant and anti-inflammatory activity, and the demonstration of comparable efficacy to standard compounds. Limitations include the *in vitro* nature of the experiments, which may not fully capture *in vivo*

Table 1: Intra- and Inter-Group Comparison of Test and Standard Groups Across Increasing Concentrations Using BSA, Egg Albumin Denaturation, DPPH, and FRAP Assays.

Assay	Group	10 µg/mL	20 µg/mL	30 µg/mL	40 µg/mL	50 µg/mL	*p*-value[a]
BSA	Test	39.22 ± 5.45	46.25 ± 6.45	54.05 ± 5.41	62.41 ± 5.23	70.19 ± 5.79	0.002*
	Standard	36.55 ± 3.78	44.96 ± 9.37	51.56 ± 4.11	59.10 ± 3.47	68.84 ± 2.79	0.003*
	p-value[b]	0.167	0.622	0.222	0.379	0.324	–
Egg albumin denaturation	Test	28.63 ± 4.33	34.19 ± 4.91	41.44 ± 3.98	48.45 ± 3.76	53.74 ± 3.79	0.000*
	Standard	28.62 ± 4.22	31.87 ± 6.05	40.50 ± 2.70	50.16 ± 4.10	54.46 ± 2.44	0.001*
	p-value[b]	0.680	0.989	0.365	0.906	0.221	–
DPPH	Test	29.34 ± 4.76	32.76 ± 4.75	41.29 ± 4.33	48.42 ± 4.28	55.12 ± 4.76	0.001*
	Standard	30.48 ± 2.73	29.76 ± 5.16	42.93 ± 4.40	48.03 ± 3.95	55.89 ± 2.62	0.002*
	p-value[b]	0.128	0.394	0.942	0.573	0.713	–
FRAP	Test	0.27 ± 0.05	0.35 ± 0.04	0.43 ± 0.04	0.53 ± 0.01	0.62 ± 0.04	0.000*
	Standard	0.32 ± 0.05	0.32 ± 0.05	0.48 ± 0.07	0.52 ± 0.01	0.64 ± 0.06	0.001*
	p-value[b]	0.281	0.904	0.393	0.609	0.384	–

[a]One-way ANOVA; [b]Independent *t*-test; *p-value < 0.05 (Statistically Significant)

pharmacokinetics, metabolism, or tissue interactions. The specific release kinetics and molecular interactions of the bioactive compounds within the gelatin matrix were also not investigated.

Future studies should focus on *in vivo* validation, mechanistic studies of the synergistic effects of the dual-botanical formulation, and optimization of the gelatin matrix to enhance stability and controlled release. Additionally, exploring crosslinking strategies or incorporating other natural polymers could further improve therapeutic potential. Overall, this study provides a solid foundation for the development of natural, plant-based, gelatin-supported bio composites as potential anti-inflammatory and antioxidant therapeutic agents.

CONCLUSION

The gelatin-based bio composite containing *Moringa oleifera* and *Camellia sinensis* exhibits strong antioxidant and anti-inflammatory activity, comparable to standard compounds. This first-of-its-kind formulation demonstrates potential as a natural therapeutic platform for inflammation-related applications. Further *in vivo* studies are needed to confirm efficacy and optimize clinical use.

ABBREVIATIONS

BSA: Bovine Serum Albumin; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **FRAP:** Ferric Reducing Antioxidant Power; **EGCG:** Epigallocatechin Gallate.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study developed a gelatin-based bio composite enriched with *Moringa oleifera* and *Camellia sinensis* extracts to harness their natural antioxidant and anti-inflammatory properties. The composite exhibited concentration-dependent efficacy in BSA, egg albumin, DPPH, and FRAP assays. Its performance was comparable to standard reference compounds, showing no significant inter-group differences. These findings underscore its promise as a biofunctional material for managing inflammation and oxidative stress in regenerative applications.

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