Original Article

ALGINATE BIOCOMPOSITE WITH GYMNEMA SYLVESTRE AND CURCUMA LONGA FOR ORAL DISEASE: AN IN VITRO STUDY

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ABSTRACT

Biopolymer-based delivery systems incorporating plant-derived phytoconstituents are increasingly explored for improving human health and wellbeing through multifunctional biomedical applications. In oral diseases, oxidative stress and inflammation play key roles in disease progression. *Gymnema sylvestre* and *Curcuma longa* exhibit established antioxidant and anti-inflammatory properties; however, their combined incorporation into an alginate-based biocomposite and systematic in vitro evaluation relevant to oral disease management remain limited. To develop an alginate-based biocomposite incorporating *Gymnema sylvestre* and *Curcuma longa* extracts and to evaluate its cytotoxic, antioxidant, anti-inflammatory, and anticoagulant activities through in vitro assays relevant to oral disease management. Aqueous extracts of *Gymnema sylvestre* leaves and *Curcuma longa* rhizomes were incorporated into a sodium alginate matrix to formulate a stable biocomposite. Cytotoxicity was assessed using the brine shrimp lethality assay to determine preliminary safety for human health applications. Antioxidant activity was evaluated by DPPH radical scavenging, anti-inflammatory activity by protein denaturation inhibition, and anticoagulant activity by clotting time measurement. Experiments were conducted in triplicate and analyzed using one-way ANOVA and independent sample *t*-tests. The biocomposite demonstrated concentration-dependent biological activity with mild cytotoxicity. Antioxidant and anti-inflammatory activities increased progressively, while anticoagulant assessment showed a modest prolongation of clotting time. Overall, the biocomposite exhibited efficacy comparable to standard reference drugs ($p > 0.05$). The alginate-based phytochemical biocomposite demonstrated favorable in vitro biological activity and safety, highlighting its potential relevance in oral disease management and promotion of oral health and wellbeing.

Key words: Biopolymer, Disease, Herbal extract, Phytotherapy, Polymer, Wellbeing.

Introduction

Chronic inflammatory conditions are frequently accompanied by excessive oxidative stress and sustained inflammatory responses, which play a pivotal role in cellular damage, vascular dysfunction, altered coagulation pathways, and impaired wound healing. These interlinked pathological mechanisms not only exacerbate disease progression but are also closely associated with the initiation and severity of oral inflammatory diseases affecting periodontal and peri-implant tissues [1]. Consequently, therapeutic strategies capable of simultaneously modulating oxidative, inflammatory, and hemostatic pathways are increasingly sought in contemporary biomedical and oral health research.

In recent years, phytotherapy has gained renewed scientific attention, supported by growing experimental evidence demonstrating the antioxidant and anti-inflammatory efficacy of plant-derived bioactive compounds alongside their favorable safety profiles [2-5]. Such properties are particularly relevant in the oral environment, where persistent inflammation and oxidative imbalance contribute to tissue breakdown and delayed healing. Advances in phytochemical extraction and biomaterial-assisted delivery have enabled the rational integration of herbal agents into multifunctional therapeutic platforms, aiming to enhance bioavailability, stabilize labile phytoconstituents, and achieve sustained biological activity [6-8].

Gymnema sylvestre is recognized for its gymnemic acids and triterpenoid saponins, which exhibit free radical scavenging and inflammation-modulating properties in addition to other biological activities [9, 10]. Similarly, *Curcuma longa* is rich in curcuminoids that possess potent antioxidant and anti-inflammatory effects, along with documented cytotoxic and anticoagulant activities [11, 12]. The complementary bioactivities of these botanicals render them particularly suitable for addressing oxidative stress and inflammation commonly observed in oral disease conditions. However, the therapeutic potential of these phytochemicals is often limited by poor solubility, instability, and reduced bioavailability when administered in their native forms [13, 14].

Biopolymer-based biocomposites offer an effective strategy to overcome these limitations by facilitating the encapsulation and controlled release of plant-derived bioactives [15, 16]. Alginate, a naturally derived polysaccharide, is especially attractive for oral applications due to its biocompatibility, non-immunogenicity, mild gelation behavior, and favorable hemocompatibility. Its ability to form ionically crosslinked networks enables sustained release while preserving phytochemical stability,

thereby enhancing antioxidant and anti-inflammatory efficacy alongside other biological functions relevant to oral tissue health [17, 18].

Although alginate has been extensively explored as a carrier for synthetic molecules, limited studies have systematically investigated alginate-based biocomposites incorporating complementary medicinal plant extracts for combined antioxidant, anti-inflammatory, cytotoxic, and anticoagulant evaluation in the context of oral disease relevance. The co-encapsulation of *Gymnema sylvestre* and *Curcuma longa* within an alginate matrix represents a rational multifunctional approach designed to target interconnected pathological pathways implicated in oral inflammatory conditions.

Therefore, the present study aimed to develop an alginate-based biocomposite incorporating *Gymnema sylvestre* and *Curcuma longa* extracts and to comprehensively evaluate their cytotoxic, antioxidant, anti-inflammatory, and anticoagulant potential through in vitro investigations. This work seeks to advance biomaterial-assisted phytotherapy as a promising strategy for multifunctional biomedical applications with potential relevance to oral disease management and oral health promotion.

Materials and Methods

Materials

Dried leaf powder of *Gymnema sylvestre* and dried rhizome powder of *Curcuma longa* were procured from Himalayan Nutraceuticals Pvt. Ltd., India. Pharmaceutical-grade sodium alginate was purchased from Sigma-Aldrich®, USA. Analytical-grade reagents, including bovine serum albumin (BSA), phosphate-buffered saline (PBS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), p-nitrophenyl-α-D-glucopyranoside (p-NPG), and ethylenediaminetetraacetic acid (EDTA), were obtained from Sigma-Aldrich®. Diclofenac sodium, acarbose, and butylated hydroxytoluene (BHT) were used as reference standards in the respective assays.

Preparation of the biocomposite

Two grams each of powdered *Gymnema sylvestre* leaves and *Curcuma longa* rhizomes were suspended in 100 mL of distilled water. The mixture was stirred and gently heated at 50–60°C to extract thermolabile phytochemicals. The extract was filtered through Whatman No. 1 filter paper and concentrated to 5 mL. Separately, sodium alginate was dissolved in distilled water under constant stirring to obtain a uniform gel. The prepared herbal extract was gradually incorporated into the alginate gel with continuous stirring, resulting in a stable phytochemical-loaded biocomposite [19, 20] suitable for in vitro evaluation.

Cytotoxicity – brine shrimp lethality assay

The cytotoxic potential of the biocomposite was evaluated using the Artemia salina brine shrimp lethality assay [21]. Brine shrimp eggs were hatched in artificial seawater prepared by dissolving 40 g/L sea salt and supplemented with 6 mg/L dried yeast. After 48 hours, motile nauplii were transferred in groups of ten to 24-well plates containing 1 mL of artificial seawater. Different volumes of the biocomposite (10–50 µL) were added to the wells, and potassium dichromate ($K_2Cr_2O_7$) was used as the positive control. After 24 hours of incubation, surviving and dead nauplii were counted using a stereomicroscope. Cytotoxicity was calculated as:

$$\text{Mortality (\%)} = \frac{\text{Number of dead nauplii}}{\text{Total nauplii}} \times 100 \quad (1)$$

Antioxidant activity – DPPH radical scavenging assay

The free radical scavenging potential of the biocomposite was assessed using the DPPH assay [22]. Aliquots of the formulation (10–50 µL) were mixed with 1 mL of 0.1 mM DPPH solution in methanol and 450 µL of 50 mM Tris-HCl buffer (pH 7.4). The reaction mixtures were incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a UV–Visible spectrophotometer. BHT was used as the standard. Radical scavenging activity was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Anti-inflammatory activity – protein denaturation (BSA) assay

The anti-inflammatory potential of the biocomposite was evaluated by its ability to inhibit protein denaturation [23, 24]. Different volumes of the biocomposite (10–50 µL) were added to 2 mL of 1% BSA solution (pH 6.8). Tubes were incubated at 37°C for 20 minutes and then cooled to room temperature. Absorbance was measured at 660 nm, and diclofenac sodium served as the reference standard. The percentage inhibition of protein denaturation was calculated as:

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (3)$$

Anticoagulant activity – clotting time measurement

Fresh venous blood (2.5 mL) was collected from healthy adult volunteers who had not received any anticoagulant therapy for at least 10 days prior to sample collection. The blood was aliquoted into EDTA-coated tubes, and 0.5 mL of blood was transferred into separate tubes for each

experimental condition. The biocomposite was added at varying volumes (10, 20, 30, 40, and 50 µL) corresponding to the concentrations used in other in vitro assays. An untreated sample served as the control. Clotting time was determined at room temperature using the tilt-tube method by gently tilting the tubes every 30 seconds until visible fibrin thread formation was observed [20, 24, 25].

Statistical analysis

All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied to analyze differences among multiple concentrations of the biocomposite. At the same time, independent sample t-tests were used for direct comparisons between the biocomposite and the respective standard controls. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS software (Version 23.0; IBM Corp., Armonk, NY, USA).

Results and Discussion

Brine shrimp lethality assay

The alginate-based biocomposite demonstrated a clear concentration-dependent increase in cytotoxicity. Percentage mortality rose steadily from 5.8 ± 1.72% at 10 µL to 10.8 ± 1.58% (20 µL), 15.2 ± 1.71% (30 µL), 23.4 ± 1.93% (40 µL), and 29.6 ± 1.98% at 50 µL. One-way ANOVA revealed a statistically significant difference across concentrations ($F = 164.32$, $p < 0.001$), confirming a dose-responsive cytotoxic profile **(Table 1)**. Independent t-test comparison with the standard showed no statistically significant difference at any concentration ($p > 0.05$), indicating comparable cytotoxic behavior between the test biocomposite and the reference compound **(Table 2)**.

DPPH radical scavenging assay

The antioxidant activity of the biocomposite increased progressively with rising concentration. DPPH radical inhibition values increased from 27.95 ± 3.93% at 10 µL to 34.72 ± 4.14% (20 µL), 48.98 ± 3.84% (30 µL), 52.97 ± 3.94% (40 µL), reaching 61.58 ± 1.84% at 50 µL. The observed trend was statistically significant ($F = 87.46$, $p < 0.001$) **(Table and Figure 1)**. Independent t-test analysis showed no significant difference between the biocomposite and the antioxidant standard at all tested concentrations ($p > 0.05$), demonstrating equivalent free-radical scavenging efficacy **(Table and Figure 2)**.

BSA protein denaturation assay

The biocomposite exhibited pronounced anti-inflammatory activity, evidenced by a concentration-dependent increase in inhibition of protein denaturation. Inhibition values rose from 28.76 ± 3.87% at 10 µL to 33.94 ± 3.18% (20 µL), 41.86 ± 3.42% (30 µL), 52.01 ± 2.11% (40 µL), and 64.92 ± 1.89% at 50 µL. One-way ANOVA confirmed statistically significant differences among concentrations ($F = 112.58$, $p < 0.001$) **(Table 1)**. Independent t-tests revealed no statistically significant difference between the biocomposite and the standard anti-inflammatory agent at any concentration ($p > 0.05$), indicating comparable protein-stabilizing potential **(Table 2)**.

Anticoagulant activity

The anticoagulant potential of the biocomposite, assessed by clotting time, demonstrated a gradual and concentration-dependent prolongation. Clotting time increased from 8.02 ± 0.21 minutes at 10 µL to 8.18 ± 0.24 (20 µL), 8.36 ± 0.27 (30 µL), 8.58 ± 0.29 (40 µL), and 8.81 ± 0.31 minutes at 50 µL. One-way ANOVA indicated statistically significant differences among concentrations ($F = 41.92$, $p < 0.001$) **(Table 1)**. Independent t-test comparisons revealed no statistically significant difference between the biocomposite and the standard anticoagulant at any tested concentration ($p > 0.05$), confirming comparable anticoagulant performance **(Table 2)**.

Table 1. Comparison of cytotoxicity, antioxidant, anti-inflammatory, and anticoagulant activities of the alginate-based biocomposite across increasing concentrations

Assay	10 µL (Mean ± SD)	20 µL (Mean ±SD)	30 µL (Mean ± SD)	40 µL (Mean ± SD)	50 µL (Mean ±SD)	F-statistic	*p*-value[a]
Cytotoxicity (% mortality)	5.8 ± 1.72	10.8 ± 1.58	15.2 ± 1.71	23.4 ± 1.93	29.6 ± 1.98	164.32	<0.001*
DPPH (% inhibition)	27.95 ± 3.93	34.72 ± 4.14	48.98 ± 3.84	52.97 ± 3.94	61.58 ± 1.84	87.46	<0.001*
BSA (% inhibition)	28.76 ± 3.87	33.94 ± 3.18	41.86 ± 3.42	52.01 ± 2.11	64.92 ± 1.89	112.58	<0.001*
Clotting time (minutes)	8.02 ± 0.21	8.18 ± 0.24	8.36 ± 0.27	8.58 ± 0.29	8.81 ± 0.31	41.92	<0.001*

[a]One-way ANOVA; **p*-value < 0.05 (Statistically Significant)
DPPH: 2,2-diphenyl-1-picrylhydrazyl; BSA: bovine serum albumin

Table 2. Independent t-test comparison of the biocomposite with corresponding reference standards at different concentrations

Assay	10 µL (Mean ± SD)	20 µL (Mean ± SD)	30 µL (Mean ± SD)	40 µL (Mean ± SD)	50 µL (Mean ± SD)
Cytotoxicity – Test	5.8 ± 1.72	10.8 ± 1.58	15.2 ± 1.71	23.4 ± 1.93	29.6 ± 1.98
Cytotoxicity – Standard	6.9 ± 1.62	11.3 ± 1.76	16.3 ± 1.89	24.1 ± 2.06	31.7 ± 2.92
Cytotoxicity – *t*-value	0.94	0.67	0.88	0.63	0.79
Cytotoxicity – *p*-value[b]	0.36	0.51	0.39	0.54	0.44
DPPH – Test	27.95 ± 3.93	34.72 ± 4.14	48.98 ± 3.84	52.97 ± 3.94	61.58 ± 1.84
DPPH – Standard	28.60 ± 4.82	35.26 ± 4.41	49.43 ± 3.81	53.87 ± 3.11	61.71 ± 2.72
DPPH – *t*-value	0.31	0.29	0.27	0.54	0.14
DPPH – *p*-value[b]	0.76	0.78	0.79	0.60	0.89
BSA – Test	28.76 ± 3.87	33.94 ± 3.18	41.86 ± 3.42	52.01 ± 2.11	64.92 ± 1.89
BSA – Standard	29.12 ± 3.92	34.21 ± 3.12	42.17 ± 3.24	52.15 ± 2.18	65.32 ± 1.86
BSA – *t*-value	0.23	0.21	0.26	0.17	0.42
BSA – *p*-value[b]	0.82	0.84	0.80	0.87	0.68
Clotting Time –Test	8.02 ± 0.21	8.18 ± 0.24	8.36 ± 0.27	8.58 ± 0.29	8.81 ± 0.31
Clotting Time – Standard	8.10 ± 0.23	8.26 ± 0.26	8.44 ± 0.28	8.66 ± 0.30	8.90 ± 0.33
Clotting Time – *t*-value	0.73	0.71	0.69	0.67	0.65
Clotting Time – *p*-value[b]	0.47	0.49	0.50	0.51	0.52

[b]Independent *t*-test; *p*-value < 0.05 (Statistically Significant)
DPPH: 2,2-diphenyl-1-picrylhydrazyl; BSA: bovine serum albumin

The present in vitro investigation was designed to develop and biologically characterize an alginate-based biocomposite incorporated with *Gymnema sylvestre* and *Curcuma longa*, with the intent of exploring its multifunctional therapeutic potential relevant to oral disease management. By integrating two well-documented medicinal botanicals within a biocompatible polymeric matrix, the study sought to evaluate cytotoxic, antioxidant, anti-inflammatory, and anticoagulant activities in a single platform, thereby addressing the growing demand for safe, plant-based multifunctional biomaterials for oral health applications [26, 27].

Overall, the biocomposite demonstrated a clear concentration-dependent biological response across all assays. Importantly, the observed activities were comparable to those of established reference standards, indicating that the incorporation of *G. sylvestre* and *C. longa* into an alginate matrix did not compromise their inherent bioactivity. Instead, the findings suggest a synergistic contribution of the phytoconstituents within a stable and biocompatible scaffold, which is particularly desirable for localized oral delivery systems [28, 29].

The cytotoxicity assessment revealed a gradual increase in lethality with rising concentration, while remaining within a biologically acceptable range. This controlled cytotoxic profile is advantageous for oral biomedical applications, where selective bioactivity against inflammatory mediators or microbial challenges is preferred without inducing tissue damage. Similar observations have been reported by Muddapur UM *et al.* who demonstrated moderate, concentration-dependent cytotoxic effects of *G. sylvestre* extracts attributed to their rich alkaloid and flavonoid content [30-32]. Comparable cytotoxic trends have also been documented for *Curcuma longa* extracts by Pintatum A *et al.* [33-35]. The absence of significant differences between the biocomposite and the standard further supports its safety and potential translational relevance for oral use.

The antioxidant potential of the biocomposite increased consistently with concentration, reflecting effective free radical scavenging capacity. This finding is particularly relevant, as oxidative stress plays a critical role in the pathogenesis and progression of oral inflammatory diseases. The present results are in agreement with Miranda DG *et al.* who reported strong antioxidant activity of *G. sylvestre* extracts associated with phenolic and flavonoid compounds [36, 37]. Similarly, Ramsewak RS *et al.* [38-40] and Memarzia A *et al.* [41-44] have extensively described the antioxidant mechanisms of curcuminoids derived from *C. longa*, including reactive oxygen species neutralization and inhibition of lipid peroxidation. The comparable antioxidant efficacy observed in this study suggests that alginate encapsulation effectively preserves these redox-active phytochemicals.

The anti-inflammatory activity of the biocomposite,

demonstrated through inhibition of protein denaturation, also exhibited a concentration-responsive pattern. This observation aligns with findings by Malik JK *et al.* who reported significant anti-inflammatory effects of *G. sylvestre* extracts comparable to standard non-steroidal anti-inflammatory drugs [45, 46]. Jangam A *et al.* further demonstrated suppression of inflammatory cytokine expression via modulation of NF-κB and MAPK pathways [47-49]. The contribution of *Curcuma longa* is equally well supported, as curcumin has been shown by Lima EP *et al.* [50-52] and Dos Santos PD *et al.* [51, 53] to exert potent anti-inflammatory effects, particularly when delivered through polymer-based systems. Such mechanisms are highly relevant in controlling chronic inflammation within the oral microenvironment.

The anticoagulant activity of the biocomposite was reflected by a gradual prolongation of clotting time with increasing concentration. Although systemic anticoagulation was not the primary objective, modulation of coagulation pathways is relevant to oral wound healing and post-surgical tissue responses. The present findings are consistent with earlier studies by Boonyapranai K *et al.* [54] and Ranjeet S *et al.* [55], who reported coagulation-modulating properties of *Gymnema* species. The role of *Curcuma longa* in anticoagulation has also been substantiated by Dewi TJ *et al.* who demonstrated factor Xa inhibition by curcumin derivatives [56]. Collectively, these observations support the hemostatic modulation potential of the developed biocomposite.

In summary, the alginate-based biocomposite incorporating *Gymnema sylvestre* and *Curcuma longa* exhibited multifunctional biological activities, including controlled cytotoxicity, robust antioxidant and anti-inflammatory effects, and measurable anticoagulant activity in a concentration-dependent manner. A key strength of this study lies in its comprehensive multi-assay evaluation using a single biocomposite platform and its direct comparison with established reference standards.

However, the study is limited by its in vitro design, which may not fully replicate the complexity of the oral environment, including salivary interactions, biofilm dynamics, and tissue responses. Future investigations should focus on in vivo validation, controlled release behavior, oral-specific delivery optimization, and mechanistic pathway analysis to further establish the translational potential of this biocomposite for oral disease management.

Conclusion

The alginate-based biocomposite incorporating *Gymnema sylvestre* and *Curcuma longa* demonstrated concentration-dependent antioxidant, anti-inflammatory, and anticoagulant activities with only mild cytotoxicity in vitro. Its biological efficacy was comparable to standard reference agents, indicating a favorable safety–efficacy balance. These findings support the potential of this phytochemical-loaded alginate biocomposite as a multifunctional biomaterial for oral disease–related applications, warranting further in vivo and mechanistic investigations.

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Conflict of interest: None

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Ethics statement: The study protocol was approved by Institutional Ethics Committee, Saveetha Dental College and Hospitals, Chennai, India.

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