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Original Article

Phytochemical-Infused Chitosan Biocomposites: Cytotoxic, Antidiabetic, and Anticoagulant Potential of *Vaccinium* and *Zingiber* Extracts

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ABSTRACT

Background: The integration of plant-derived bioactives with natural polymers offers a sustainable approach to therapeutic biomaterials. *Vaccinium sect. Cyanococcus* (blueberry) and *Zingiber officinale* (ginger) are known for their antidiabetic and anticoagulant properties, while chitosan serves as a biodegradable, biocompatible carrier. **Aim:** To develop a chitosan-based biocomposite loaded with *V. cyanococcus* and *Z. officinale* extracts and evaluate its *in vitro* antidiabetic, anticoagulant, and cytotoxic activities. **Materials and Methods:** Biocomposites were prepared by incorporating aqueous extracts of *V. cyanococcus* and *Z. officinale* into a chitosan matrix. Antidiabetic activity was assessed via α-amylase and α-glucosidase inhibition assays. Anticoagulant activity was evaluated using clotting time analysis. Biocompatibility was tested via the brine shrimp lethality assay. Data were analyzed statistically, with significance set at $p<0.05$. **Results:** The biocomposite showed significant, dose-dependent inhibition of α-amylase (48.03-85.09%) and α-glucosidase (47.03-84.04%) activities, comparable to the standard drug acarbose ($p>0.05$). Clotting time in the treated group (8.40±0.10 min) showed a mild, non-significant decrease compared to control (8.60±0.10 min). Cytotoxicity was minimal at lower concentrations, with nauplii viability remaining above 60% even at the highest dose after 48 hr. **Conclusion:** The chitosan-based biocomposite enriched with *V. cyanococcus* and *Z. officinale* exhibits promising antidiabetic and mild anticoagulant activity with acceptable biocompatibility. These findings support its potential use in managing metabolic and thrombotic conditions.

Keywords: Biocomposite, Enzymatic inhibition, Glycemic modulation, Herbal bioactives, Natural polysaccharides, Plant-derived compounds, Therapeutic biomaterials.

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INTRODUCTION

The convergence of natural polymers and bioactive phytoconstituents in the development of composite materials has drawn growing interest, particularly in the realm of biomedical and therapeutic innovations.[1] Biocomposites-composed of naturally sourced polymers integrated with plant-derived bioactives-are emerging as environmentally friendly and functionally superior substitutes to traditional synthetic materials, offering key benefits such as biodegradability, inherent biocompatibility, and minimal ecological footprint.[2] These composites hold immense potential in fields that demand enhanced biological performance and regulated release of therapeutic agents, notably in wound healing, targeted drug delivery, and tissue regeneration. Moreover, the incorporation of phytochemicals with well-documented therapeutic efficacy into such biocomposites presents promising strategies for addressing widespread chronic health conditions, including metabolic syndromes and thrombotic pathologies.[3]

Chitosan, a naturally occurring polysaccharide produced via the deacetylation of chitin, serves as a foundational biopolymer in the fabrication of such composites. Extracted predominantly from crustacean shells, chitosan is lauded for its favorable characteristics, including biodegradability, non-toxic nature, film-forming capacity, and its affinity for interacting with diverse bioactive molecules.[4] In addition, it exhibits intrinsic antimicrobial effects and pH-responsive behavior, making it an exemplary carrier for herbal extracts with synergistic therapeutic effects.[5] The incorporation of botanicals into biomaterial matrices is gaining traction in medical research, driving efforts to evaluate various plant-based formulations through both *in vitro* and *in vivo* models.[6-9]

Among the array of plant bioactives, *Vaccinium sect. Cyanococcus* (commonly known as blueberry) and *Zingiber officinale*

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(ginger) are notable for their broad-spectrum pharmacological actions. Blueberries are a rich source of anthocyanins and flavonoids, compounds known for their potent antioxidant, anti-inflammatory, and insulin-sensitizing properties.[10] These attributes render them particularly effective in glycemic regulation, thereby supporting their inclusion in biomaterials aimed at diabetes management.[11] Ginger, on the other hand, contains therapeutically significant constituents such as gingerols and shogaols, which have demonstrated marked antidiabetic and anticoagulant activities.[12] The co-delivery of these extracts in a biocomposite system introduces a synergistic platform with amplified therapeutic value.

The integration of blueberry and ginger extracts into a chitosan matrix presents an innovative strategy for fabricating multifunctional biocomposites that exhibit promising antidiabetic and anticoagulant efficacy. Encapsulation within the chitosan framework ensures both protection and controlled release of these sensitive bioactives, thereby facilitating targeted and sustained therapeutic action. Such formulations are particularly advantageous in addressing complications associated with diabetes and cardiovascular disorders. Furthermore, leveraging biodegradable and renewable resources in biocomposite design aligns with the growing pursuit of sustainable and environmentally responsible medical technologies, diminishing the dependence on synthetic drugs that often contribute to ecological burden.

This study endeavors to develop and characterize a chitosan-based biocomposite embedded with blueberry and ginger extracts, with an emphasis on evaluating its cytotoxicity, antidiabetic, and anticoagulant activities through a series of *in vitro* experimental assays.

MATERIALS AND METHODS

Preparation of Chitosan-Based Biocomposite Enriched with *Vaccinium sect. Cyanococcus* and *Zingiber officinale* Extracts

To develop the biocomposite, 2 grams each of dried *Zingiber officinale* (ginger) and *Vaccinium sect. Cyanococcus* (blueberry) powders were extracted in 100 mL of distilled water. The extraction process was carried out at 50-60°C to optimize the release of bioactive constituents while preserving thermolabile compounds. The mixture was thoroughly stirred, filtered to remove particulates, and concentrated to approximately 5 mL. In parallel, chitosan was dissolved in aqueous acetic acid with constant stirring to obtain a uniform polymeric matrix. The concentrated plant extract was gradually incorporated into the chitosan solution under continuous agitation to ensure homogenous dispersion and encapsulation of the phytoconstituents.[13] The resulting formulation-a chitosan-based biocomposite enriched with blueberry and ginger extracts-was subsequently subjected to biological assessments.

Evaluation of Antidiabetic Activity

The antidiabetic potential of the biocomposite was assessed via standard enzymatic inhibition assays targeting α-amylase and α-glucosidase. Each assay was conducted in triplicate, and the mean values were used for statistical evaluation.

α-Amylase Inhibitory Assay

Reaction mixtures (1 mL) comprising 500 µL of 100 mM phosphate buffer (pH 6.8), 100 µL of porcine pancreatic α-amylase (2 U/mL), and varying concentrations of the biocomposite (10-50 µg/mL) were pre-incubated at 37°C for 20 min. Enzymatic activity was initiated by adding 200 µL of 1% (w/v) soluble starch. After a 30-min incubation, the reaction was terminated with 1 mL of 3,5-dinitrosalicylic acid (DNS) reagent, followed by heating in a boiling water bath for 10 min. Absorbance was measured at 540 nm using a microplate reader (Multiskan, Thermo Scientific, Version 1.00.40). Acarbose (10-50 µg/mL) served as the positive control, and phosphate buffer was used as the negative control. Inhibitory activity was calculated using:

$$\text{Inhibition } (\%) = [(Ac - As)/Ac] \times 100$$

Where Ac=control absorbance and As=sample absorbance.

α-Glucosidase Inhibitory Assay

Reaction mixtures included 500 µL of 100 mM phosphate buffer (pH 6.8), 100 µL of α-glucosidase enzyme (1 U/mL), and biocomposite concentrations ranging from 10-50 µg/mL. After 15 min of pre-incubation at 37°C, 200 µL of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG) was added. After 20 min, the reaction was stopped with 50 µL of 0.1 M sodium carbonate. Absorbance was read at 405 nm. Acarbose served as a positive control and buffer-only as the negative control. The percentage of enzyme inhibition was determined accordingly.

Evaluation of Anticoagulant Activity

Anticoagulant efficacy was evaluated by measuring clotting time. Fresh venous blood (2.5 mL) was collected aseptically from healthy adult volunteers free from anticoagulant medication for the prior 10 days. Blood was aliquoted into EDTA-coated tubes and divided into control and experimental groups. For the experimental group, 0.5 mL of blood was mixed with 100 µL of the biocomposite. The control group received no treatment. All samples were monitored at room temperature, and clotting time was recorded from sample collection to the appearance of visible fibrin strands by tilting the tubes at 30-sec intervals. Triplicates were used for each group.

Biocompatibility Assessment

Brine Shrimp Lethality Assay

Biocompatibility was tested using *Artemia salina* larvae. Artificial seawater was prepared by dissolving 2 g of iodine-free salt in

200 mL distilled water. Nauplii were introduced into wells of ELISA plates (10 larvae/well) containing various biocomposite concentrations (5-80 µg/mL). Controls received saline alone. Plates were kept under constant illumination at ambient temperature. After 24 hr, live and dead nauplii were counted using a stereomicroscope (Metkorp Zoom-vi). Mortality was assessed via:[13]

$$\% \text{ Mortality} = (\text{Dead nauplii} / \text{Total nauplii}) \times 100$$

The assay was performed in triplicate.

Statistical Analysis

Data were analyzed using SPSS software (Version 23.0). Statistical significance was determined using independent t-tests and ANOVA. A p-value <0.05 was considered statistically significant.

RESULTS

α-Amylase Inhibitory Assay

The biocomposite exhibited a dose-dependent increase in α-amylase inhibition, rising from 48.03±1.12% at 10 µg/mL to 85.09±1.15% at 50 µg/mL (Figure 1). Although acarbose showed slightly superior inhibition at each concentration, the difference was statistically non-significant (p>0.05), highlighting the biocomposite's strong inhibitory potential.

α-Glucosidase Inhibitory Assay

Similar trends were observed in α-glucosidase inhibition. The biocomposite demonstrated increasing inhibition from 47.03±2.03% to 84.04±1.07% across the 10-50 µg/mL range (Figure 2). Though marginally lower than acarbose, the inhibitory values were not significantly different (p>0.05), affirming potent α-glucosidase inhibition.

Anticoagulant Activity

The control group showed a clotting time of 8.60±0.10 min, which is within normal physiological range (8-15 min). The experimental group exhibited a marginally reduced clotting time of 8.40±0.10 min. However, this reduction was statistically insignificant (p>0.05), indicating that the biocomposite did not alter coagulation pathways substantially (Table 1).

Brine Shrimp Lethality Assay

No acute cytotoxicity was observed on Day 1 at all tested concentrations. However, by Day 2, nauplii viability decreased at concentrations ≥20 µg/mL, with a viability of 60±1% at 80 µg/mL (Figure 3). The control group maintained 100% viability. These findings suggest the biocomposite remains biocompatible at lower concentrations.

DISCUSSION

The present investigation aimed to formulate and evaluate a novel chitosan-based biocomposite infused with *Vaccinium sect. Cyanococcus* (blueberry) and *Zingiber officinale* (ginger) extracts, focusing on its antidiabetic, anticoagulant, and biocompatibility profiles. The findings revealed that the biocomposite exhibited potent, concentration-dependent inhibitory activity against both α-amylase and α-glucosidase, suggesting its potential role in modulating hyperglycemia-a critical therapeutic target in type 2 diabetes mellitus.

The observed α-amylase and α-glucosidase inhibitory effects are likely attributable to the rich phytochemical content of the incorporated botanicals. Blueberries are abundant in polyphenolic compounds, particularly anthocyanins such as delphinidin and cyanidin glycosides, which have been shown to exert hypoglycemic effects by inhibiting carbohydrate-hydrolyzing enzymes and modulating insulin signaling pathways.[14] Additionally, *Z.*

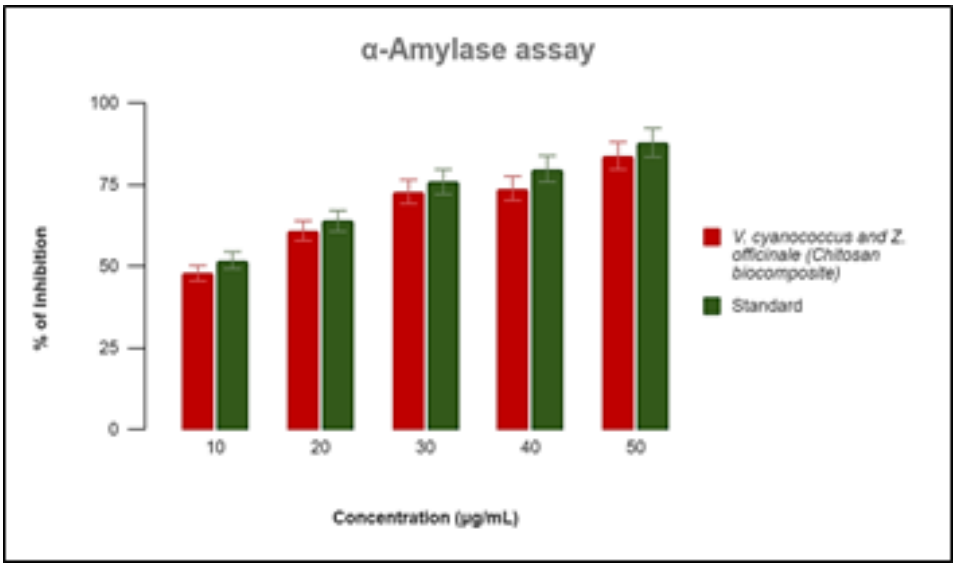

Figure 1: α-Amylase inhibition (%) of biocomposite and standard at 10-50 µg/mL.

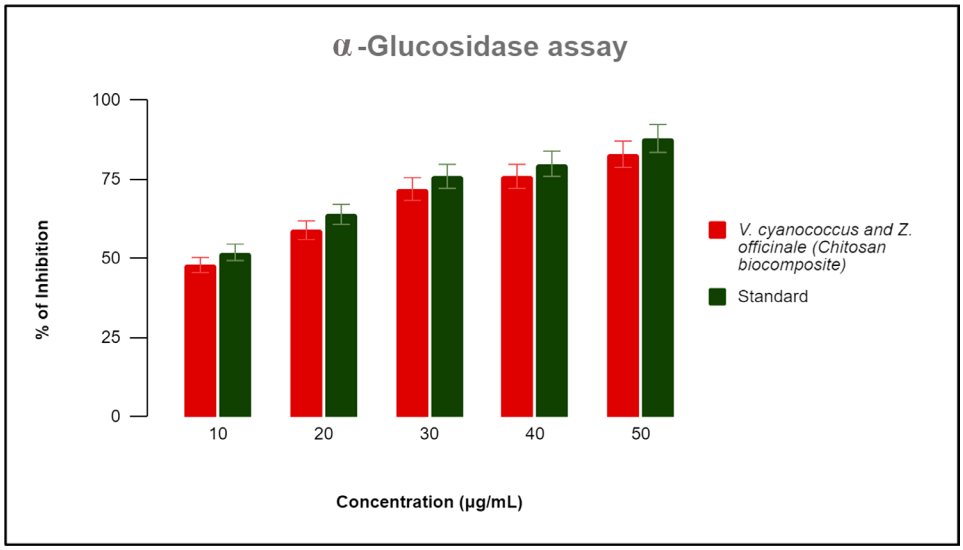

Figure 2: α-Glucosidase inhibition (%) of biocomposite and standard at 10-50 µg/mL.

Table 1: Effect of biocomposite on blood clotting time vs. control.

Group	Replicate 1 (mins)	Replicate 2 (mins)	Replicate 3 (mins)	Mean±SD (mins)	*p*-value
Control (Normal Blood)	8.5	8.7	8.6	8.60±0.10	0.158
Experimental (Biocomposite Treated)	8.2	8.5	8.5	8.40±0.10	

officinale contains bioactive constituents like 6-gingerol, shogaol, and paradol, which have demonstrated the ability to suppress α-glucosidase activity and enhance peripheral glucose uptake through AMP-activated protein kinase activation.[15] The dual inhibition of α-amylase and α-glucosidase reduces the enzymatic degradation of complex carbohydrates into glucose, thereby mitigating sharp postprandial glycemic excursions.

Chitosan, the polymeric matrix in this formulation, may further potentiate the antidiabetic efficacy by serving as a bioadhesive carrier that prolongs the gastrointestinal residence time of phytochemicals and facilitates their controlled release and mucosal absorption. Chitosan's own capacity to delay glucose absorption and modulate gut microbiota has also been documented,[16] which could synergistically enhance glycemic control.[17] A comparable study by Alghuthaymi *et al.*,[18] reported strong α-glucosidase inhibition using a chitosan-cinnamon bark composite, reinforcing the notion that the polymer matrix plays an integral role in the sustained bioactivity of herbal constituents. Likewise, Al Hunduwan *et al.*,[19] demonstrated antidiabetic effects of chitosan-fenugreek composites via improved insulin secretion. In the current study, while the standard drug acarbose exhibited marginally superior enzyme inhibition, the differences were statistically insignificant, underscoring the potential of the biocomposite as a natural, side effect-sparing alternative to conventional antidiabetic agents, which are often associated with gastrointestinal disturbances.

Regarding anticoagulant potential, the biocomposite demonstrated a slight, though statistically non-significant, prolongation of clotting time. This suggests a mild influence on the coagulation cascade. Blueberries are known to contain salicylate derivatives and flavonoids that exert antithrombotic effects by modulating platelet activation and thromboxane A2 synthesis.[20] Ginger, in particular, is well-documented for its anticoagulant and antiplatelet properties, which are primarily mediated by 6-gingerol's inhibitory action on arachidonic acid metabolism and thromboxane synthetase activity.[21] The incorporation of these agents within a chitosan matrix may lead to a sustained and controlled release, producing a subtle yet potentially clinically meaningful anticoagulant effect, especially relevant in individuals with a high risk of thromboembolic events often co-existing with diabetes.[22] A chitosan-curcumin formulation studied by Gupta *et al.*,[23] showed similar prolongation of clotting time.

The biocompatibility assessment through the brine shrimp lethality assay revealed low cytotoxicity at therapeutic concentrations, with notable nauplii survival even at higher doses on Day 1. However, prolonged exposure (48 hr) led to a dose-dependent decline in viability, particularly at concentrations exceeding 40 µg/mL. This observation underscores the importance of dose optimization in future *in vivo* applications. The biocompatibility of chitosan-based systems has been extensively reported in literature, where Iancu *et al.*,[24] illustrated similar outcomes with chitosan-*Lythri herba* films, suggesting

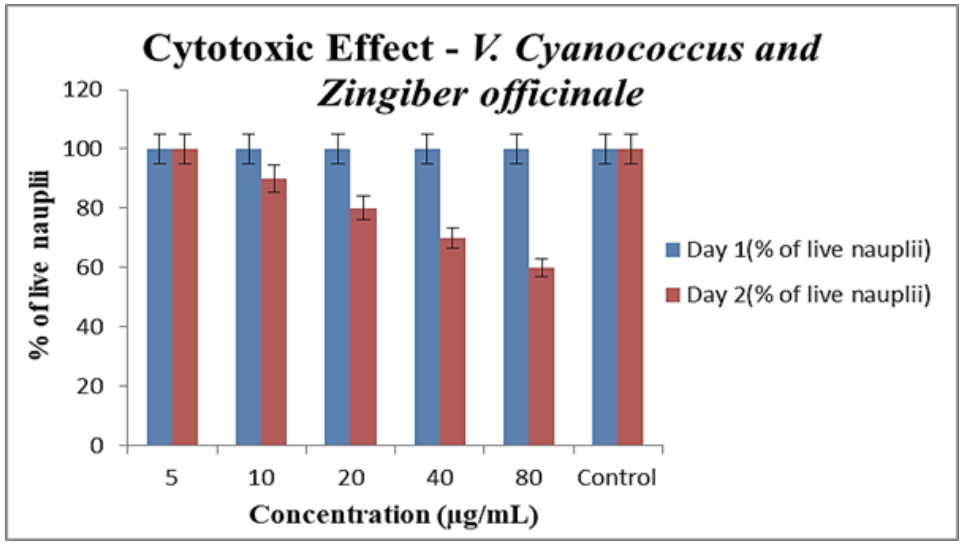

Figure 3: Cytotoxicity of biocomposite on *Artemia salina* at 5-80 µg/mL after 24 and 48 hr.

that the cytotoxic potential is largely dependent on the nature and concentration of the incorporated bioactive compounds.

Furthermore, the encapsulation of phytochemicals within the chitosan matrix may protect them from oxidative degradation and enzymatic hydrolysis, thereby enhancing their stability and therapeutic lifespan. The controlled release profile of such biocomposites can help maintain therapeutic concentrations for prolonged durations, potentially reducing dosing frequency and improving patient compliance-an important consideration in chronic diseases like diabetes. These findings align with the increasing emphasis on plant-derived nanocomposite systems in biomedical research. Several studies have explored the integration of botanical extracts into biodegradable polymers to yield multifunctional therapeutic platforms with anti-inflammatory, antioxidant, and antimicrobial properties.[25-27] The present study adds to this growing body of evidence by showcasing a dual-functioning biocomposite with significant antidiabetic and mild anticoagulant potential, coupled with acceptable biocompatibility.

However, the current study is limited by its *in vitro* nature, and the physiological relevance of the findings must be interpreted with caution. Future directions should include *in vivo* pharmacokinetic and pharmacodynamic studies, and long-term toxicity analyses in animal models. Investigating the interaction of this biocomposite with cellular targets such as insulin receptors, glucose transporters, and coagulation factors could provide deeper mechanistic insights. Additionally, nanoformulation approaches like ionic gelation or electrospraying may be explored to enhance delivery efficiency and tissue targeting.

In conclusion, the chitosan-based biocomposite developed herein represents a promising step toward the development of integrative, plant-based therapeutic systems for metabolic disorders. Its dual modulatory effects on glycemic control and coagulation, supported by its biocompatibility, warrant further exploration and optimization for potential translation into clinical applications.

CONCLUSION

The chitosan-based biocomposite formulated with *Vaccinium sect. Cyanococcus* and *Zingiber officinale* extracts exhibited significant *in vitro* antidiabetic and anticoagulant effects while maintaining biocompatibility. The synergistic interplay between the incorporated phytochemicals enhanced the therapeutic efficacy of the formulation. This biocomposite presents a promising, natural, and multifunctional platform for future applications in metabolic and cardiovascular disease management.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DNS: 3,5-Dinitrosalicylic Acid; **p-NPG:** *P*- Nitrophenyl-α-D-Glucopyranoside.

ETHICAL APPROVAL

The study protocol was approved by Institutional Ethical Committee, Saveetha Dental College and Hospitals, Chennai.

SUMMARY

A novel chitosan-based biocomposite incorporating *Vaccinium cyanococcus* (blueberry) and *Zingiber officinale* (ginger) extracts was developed to explore its therapeutic potential. The biocomposite demonstrated significant, dose-dependent inhibition of α-amylase and α-glucosidase, indicating strong antidiabetic activity. Mild anticoagulant effects were observed,

with minimal cytotoxicity. These findings highlight its potential for managing metabolic and thrombotic disorders.

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