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ANTICOAGULANT, THROMBOLYTIC, ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITY OF BROMELAIN-NANOSILVER FORMULATION

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Abstract

Background: Nanotechnology, particularly noble metal-based nanoparticles like silver, offers high biocompatibility and stability for medical applications, including antimicrobial, thrombolytic, and antidiabetic therapies. Bromelain from Ananas comosus enhances these effects by reducing platelet aggregation and inflammation, while green synthesis provides an eco-friendly alternative to traditional methods. Together, these advancements hold promise for treating conditions such as infections, thrombosis, and diabetes with improved safety and efficacy.

Aim: This study aims to assess the anticoagulant, thrombolytic, antidiabetic, and anti-inflammatory potential of a green-synthesized bromelain-incorporated silver nanocomposite for multifunctional therapeutic use.

Methods: Silver nanocomposite (AgNCs) were tested for anticoagulant and thrombolytic activities using human blood samples to observe clot formation and lysis. Antidiabetic activity was evaluated through alpha-amylase and alpha-glucosidase inhibition assays, with absorbance measured at 540 nm. Anti-inflammatory activity was assessed via protein denaturation (bovine and egg albumin) and membrane stability assays, with absorbance recorded at 660 nm and 560 nm, respectively.

Results: Bromelain-AgNCs showed anticoagulant activity with clotting times from 13 min (10 µg/mL) to 8 min (50 µg/mL). Thrombolytic activity increased with concentration, peaking at 20 min clot lysis at 50 µg/mL. Antidiabetic activity showed 68% α-amylase and 66% α-glucosidase inhibition at 50 µg/mL. Anti-inflammatory activity reached 77% (BSA), 77% (EA), and 85.7% (MSA) inhibition at 50 µg/mL.

Conclusion: This study demonstrates the successful synthesis of bromelain-incorporated silver nanocomposites (AgNCs) with strong multifunctional properties, including anticoagulant, thrombolytic, antidiabetic, and anti-inflammatory effects. The nanocomposites showed minimal toxicity and hold promise for safe, eco-friendly use in therapeutic applications such as wound healing, drug delivery, and the management of diabetes, inflammation, and clot-related disorders.

Keywords: Ananas comosus, Bromelain-nanosilver formulation, anticoagulant activity, thrombolytic activity, antidiabetic activity.

INTRODUCTION

The design, production, and application of materials with a nanometre as their fundamental unit of measurement are all included in nanotechnology. Among other things, this term encompasses chemistry, advanced materials, physics, and the biomedical and pharmaceutical sciences. High biocompatibility, stability, and the potential for large-scale production without the use of organic solvents are among the benefits of noble metal-based nanoparticles, which are crucial for medical applications and have a beneficial impact on biological systems. Various medications may be released under controlled conditions thanks to these nanoparticles (1).

According to studies, by strengthening antioxidant defences, nanoparticles (NPs) may help lower oxidative stress in conditions like atherosclerosis, where variables like high blood pressure and cholesterol encourage the production of reactive oxygen species (ROS) (2).

Many indigenous cultures have utilised Ananas comosus as a medicinal plant. Bromelain, a crude extract that contains closely related proteinases, is thought to be responsible for the plant's therapeutic qualities. In vitro and in vivo, bromelain demonstrates fibrinolytic, anti-edematous, antithrombotic, and anti-inflammatory properties (3). Bromelain lowers platelet aggregation, which lowers the risk of arterial thrombosis and embolism, according to ex vivo studies conducted on patients with myocardial infarction, stroke, or high platelet aggregation who received oral administration of the drug. Numerous in vitro and in vivo investigations subsequently supported these conclusions by showing that bromelain inhibits platelets in a dose-dependent manner (4).

Because of their potent ability to suppress microbial growth, silver nanoparticles—which are renowned for their special qualities—are regarded as a key weapon in the fight against pathogenic microbial activity (5). Since silver nanoparticles (AgNPs) have antimicrobial effects against a variety of infectious and multidrug-resistant bacteria, their exceptional antibacterial activity has garnered a lot of research and industrial interest. This makes them useful in medical and healthcare applications, where their incorporation into products like surgical tools, food handling equipment, dental products, catheters, and dressings has been thoroughly studied (6).

Microscopic blood clots are often necessary to repair vascular injuries in healthy individuals. Through proteolytic digestion, the fibrinolytic system efficiently breaks down insoluble clots into soluble fragments and repairs the endothelial surface of the damaged vessel (7). The natural remedy for excessive bleeding is blood clotting. This foretells a grave risk, though, if the clot lasts longer than necessary and blocks blood flow in the vessels that supply organs and tissues, leading to clinical conditions like heart attacks and strokes (8).

Thrombotic disorders have become a major social concern. The emphasis has now shifted to controlling and keeping platelets in an inactive state because anticoagulant and thrombolytic treatments are typically linked to severe bleeding complications (9). Debilitating conditions like arteriosclerosis, cardiovascular diseases, and strokes are frequently accompanied by thrombotic disorders. Conventional treatments for thrombosis, such as heparin, tissue plasminogen activator, urokinase, or streptokinase, have been beset by bleeding complications related to reocclusion and reinfarction (10).Green synthesis of nanoparticles mediated by plant extract has drawn interest from researchers due to its low cost and environmental benefits; the resulting nanoparticles have notable thrombolytic and anticoagulant properties (11). Bromelain (0.1 ug/mL) decreased the adherence of fluorescently labelled, bound, thrombin-stimulated platelets to the endothelial cells of the bovine aorta in vitro. Furthermore, platelet adhesion to endothelial cells was decreased to the low binding value of unstimulated platelets when platelets were preincubated with bromelain before thrombin activation (12).

The autoimmune condition known as type 1 diabetes is brought on by the death of pancreatic β-cells, which prevents the production of insulin and ultimately causes hyperglycemia. Oxidative stress and inflammation both contribute to the death of β-cells (13). Ag/CuO NCs mediated by Zingiber officinale extract demonstrated strong antidiabetic effects (14).

This study was conducted to evaluate the anticoagulant, thrombolytic, antidiabetic, and anti-inflammatory activities of a bromelain-nanosilver formulation.

MATERIALS AND METHOD

(i) Preparation of Plant Extract:

A volume of 25 mL of fresh Ananas comosus pulp was mixed with 100 mL of distilled water and heated at 60°C for 20 minutes. The mixture was subsequently filtered to remove particulate matter, and the resulting clear filtrate was collected and stored under appropriate conditions for further use.

(ii) Synthesis of Silver Nanoparticles:

2mM silver nitrate was solubulised in 80 millilitres of distilled water. To the resulting solution, 20 mL of the prepared A. comosus extract was added. The mixture was continuously stirred using a magnetic stirrer for 24 hours to facilitate the synthesis of silver nanoparticles.

(iii) Preparation of Bromelain Solution:

Bromelain (100 mg) was accurately weighed and dissolved in 1 mL of distilled water to prepare a concentrated enzyme solution.

(iv) Formulation of the Nanocomposite:

Equal volumes (1 mL each) of the bromelain solution and the silver nanoparticle suspension were combined and stirred at 450 rpm using a magnetic stirrer for 24 hours. A visible color change from clear white to light cloudy brown

indicated the successful formation of the bromelain–silver nanoparticle nanocomposite. The nanocomposite was stored appropriately for subsequent characterization and applications.

Anticoagulant activity:

The modified version of the methodology in Rajeshkumar et al.,(2024)(15). Freshly drawn human blood at room temperature was used to test the silver nanoparticles' anticoagulant properties. In this study 1 mL of human blood was mixed with different fixation of bromelain-incorporated silver nanocomposite at (10 – 50 µg/mL) and the control was added 1 mL of fresh human blood and the standard was used as EDTA in 20 µg/mL. After that, the two blood samples were left at room temperature for an additional hour to look for any discernible changes.

Thrombolytic activity:

The modified version of the methodology in Rajeshkumar et al.,(2024)(16). One drop of blood was placed onto a sterile glass slide and left to incubate at room temperature for forty-five minutes. After that clotted a drop of blood was added different fixation of bromelain-incorporated silver nanocomposites at (10 – 50 µg/mL) and the control was added 1 drop of fresh human blood. The glass slide was incubated at room temperature for 90 minutes in order to see the clot lysis. The incubation period was noted.

Antidiabetic assay:

The modified version of the methodology in Rajeshkumar et al.,(2024,2025)(17)(18). The antidiabetic activity of bromelain-incorporated silver nanocomposites was evaluated through alpha-amylase and alpha-glucosidase enzyme inhibition assays at concentrations ranging from 10 to 50 mg/mL.

Anti-inflammatory activity:

Bovine serum albumin denaturation (BSAD) assay

The methodology in Rajeshkumar et al. (2024,2025)(19)(20) was modified. The anti-inflammatory qualities of the green synthesised silver nanocomposite were assessed using three assays: the bovine serum albumin denaturation (BSAD) assay, the Egg Albumin denaturation assay and the membrane stabilization assay at different concentrations (10 - 50 µg/mL) of bromelain-mediated silver nanoparticles.

Statistical Analysis

The mean cell viability differences between the control and bromelain-incorporated silver nanoparticle-treated groups were evaluated across a number of factors using a two-way analysis of variance (ANOVA) and Tukey's multiple comparison post hoc test. Statistical analysis was carried out using GraphPad Prism 9 software (Dotmatics, Boston, Massachusetts, USA). Results with a p-value below 0.05 were considered statistically significant, and data are expressed as the mean with standard deviation (SD).

RESULTS

1. **Visual observation**

Fig. 1(a) Fig. 1(b) Fig. 1(c) Fig. 1(d) Fig. 1(e) Fig. 1(f)

Fig. 1 (a) shows plant extract, (b) depicts silver nitrate solution, (c) depicts preparation of nanoparticles, (d) depicts preparation of bromelain, (e) depicts preparation of nanocomposite, (f) depicts bromelain incorporated silver nanocomposite

A color change from pale to brown was observed during nanoparticle synthesis (Fig.1c), indicating silver nanoparticle formation. The solution turned darker brown upon incorporation of bromelain (Fig.1 f), confirming successful formation of the bromelain-silver nanocomposite.

2. UV visual observation

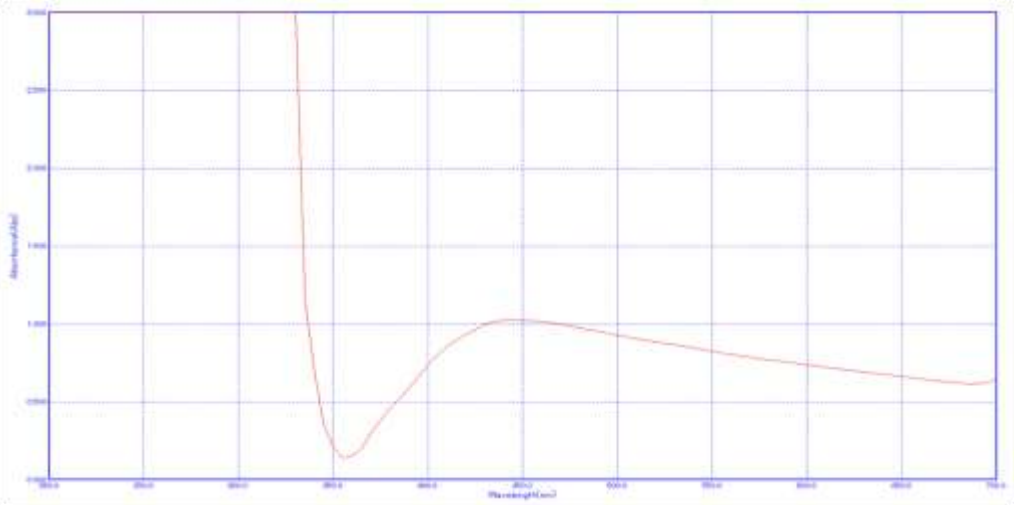

Fig. 2 : UV-Vis spectrograph

The graph shows a broad peak at 443 nm, providing preliminary confirmation of nanoparticle synthesis through UV Double beam spectrophotometer (Fig 2).

3. Anticoagulant activity

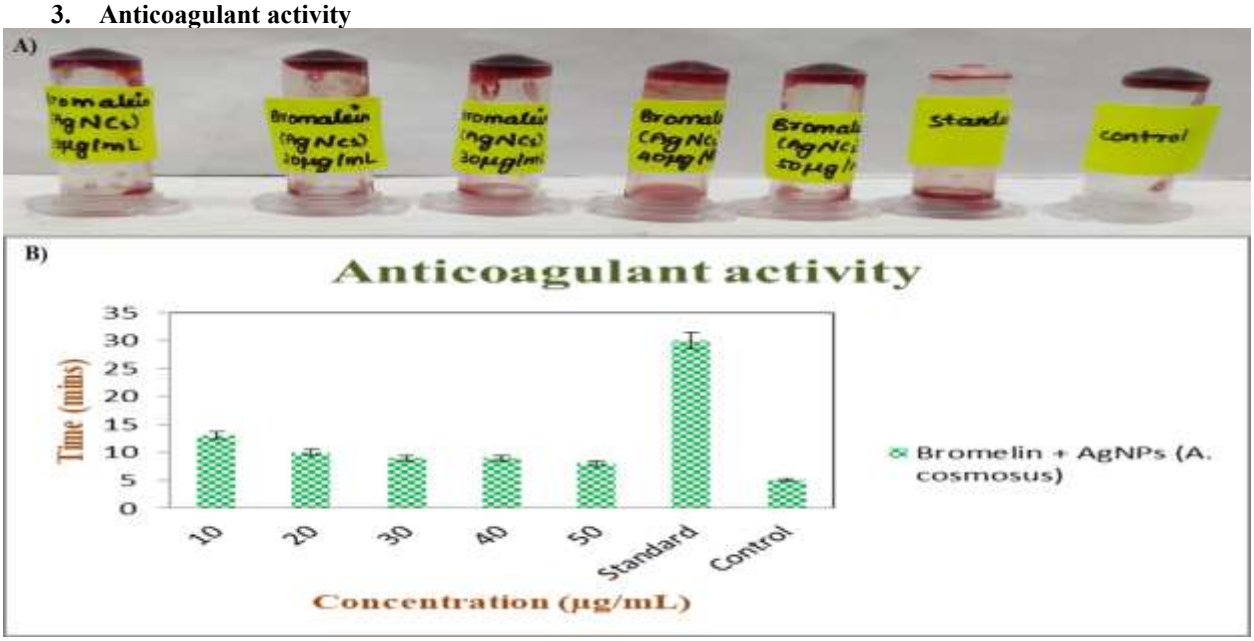

Fig 3: Anticoagulant activity of Bromelin-incorporated silver nanoparticles

In Fig. 3, the anticoagulant activity of bromelain-incorporated silver nanoparticles (AgNPs) was evaluated using blood samples at varying concentrations. The ananas comosus extract served as the control,EDTA served as the reference standard. At a concentration of 10 µg/mL, the clotting time was measured to be 13 minutes; at 20 µg/mL, it decreased to 10 minutes; and at both 30 µg/mL and 40 µg/mL, the clotting time was 9 minutes. At 50 µg/mL, the shortest clotting time among the treated groups was observed at 8 minutes. In comparison, the standard exhibited the longest clotting time at 30 minutes, while the control group had the shortest at 5 minutes. The highest clotting time among the treated samples was observed at 10 µg/mL (13 minutes), with a gradual reduction in clotting time at higher concentrations.

Visually, all samples treated with bromelain-AgNPs exhibited delayed coagulation relative to the control, indicating notable anticoagulant potential.

4. Thrombolytic activity

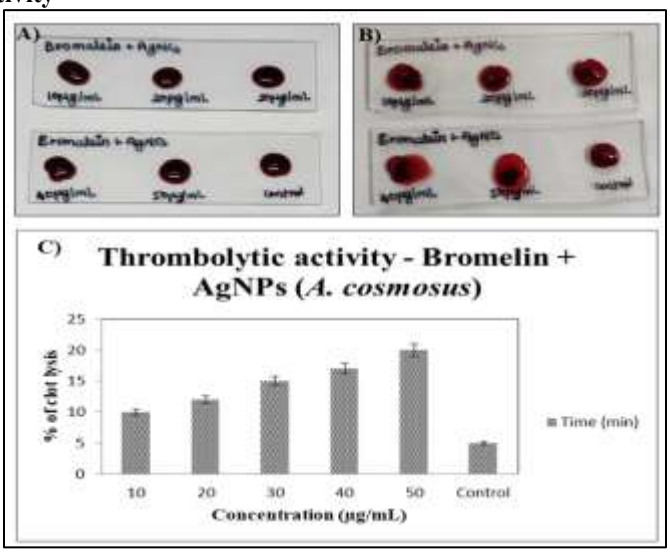

Fig 4: Thrombolytic activity of Bromelin-incorporated silver nanoparticles

In fig. 4, By comparing the incubation time needed to lyse blood clots across different doses (10–50 µg/mL)to a control group that received no treatment, Fig. 4 shows the thrombolytic activity of bromelain-incorporated silver nanoparticles. The clot lysis times observed were 10 minutes at 10 µg/mL, 12 minutes at 20 µg/mL, 15 minutes at 30 µg/mL, 17 minutes at 40 µg/mL, and a maximum of 20 minutes at 50 µg/mL. The control group showed the shortest lysis time of just 5 minutes. An increase in clot lysis time was evident, with higher concentrations of the nanocomposite demonstrating enhanced anticoagulant activity. The most pronounced effect was observed at 50 µg/mL, highlighting the strong thrombolytic potential of the bromelain-AgNPs. In contrast, the rapid clotting in the control group confirmed minimal or no anticoagulant effect in the absence of treatment.

5. Antidiabetic activity

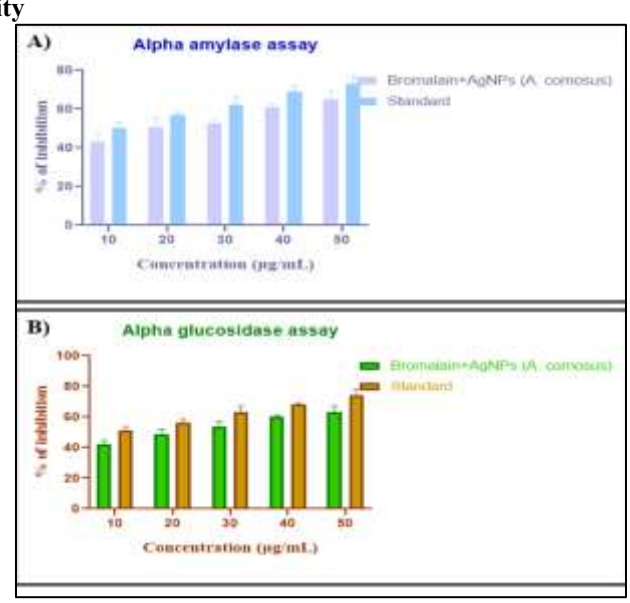

Fig 5: Antidiabetic activity of Bromelin-incorporated silver nanoparticles using A) Alpha amylase activity, and B) Alpha glucosidase activity

In Figures 5 A) and B), the antidiabetic potential of bromelain-incorporated silver nanocomposites was evaluated by assessing their inhibitory activity against two key enzymes involved in carbohydrate digestion: α-amylase and α-glucosidase. The study was conducted using various concentrations of the nanocomposite (10, 20, 30, 40, and

50 µg/mL), and Acarbose, a known antidiabetic drug, served as the standard for comparison. To ascertain the significance of the observed differences, a two-way ANOVA and Tukey's post hoc test were used in the statistical analysis. For α-amylase inhibition, the nanocomposite showed a concentration-dependent increase in enzyme inhibition. At 10 µg/mL, it exhibited 40% and 46% inhibition, while the standard showed 48% and 52%. This trend continued at 20 µg/mL, with the nanocomposite showing 47% and 54% inhibition compared to 56% and 58% for the standard. At 30 µg/mL, inhibition rose to 51% and 54% for the nanocomposite and 59% and 65% for the standard. At 40 µg/mL, the nanocomposite showed 60% and 61% inhibition, while the standard reached 67% and 71%. The highest inhibition was observed at 50 µg/mL, with the nanocomposite showing 62% and 68%, compared to 70% and 76% for the standard. Similarly, the nanocomposite demonstrated promising results in α-glucosidase inhibition. At 10 µg/mL, it showed 40% and 44% inhibition, while the standard exhibited 49% and 53%. At 20 µg/mL, the nanocomposite inhibited the enzyme by 46% and 51%, compared to 54% and 58% for the standard. At 30 µg/mL, the nanocomposite showed 51% and 56% inhibition, while the standard reached 60% and 66%. At 40 µg/mL, it achieved 61% and 59% inhibition versus 67% and 69% for the standard. At 50 µg/mL, inhibition by the nanocomposite peaked at 60% and 66%, while the standard maintained higher inhibition levels of 71% and 77%. In comparison to the standard drug Acarbose, the bromelain-incorporated silver nanocomposites consistently demonstrated slightly lower but appreciable inhibitory activity across both enzyme targets. The inhibitory effect increased progressively with concentration, indicating a dose-dependent antidiabetic response. These findings suggest that bromelain-AgNPs possess significant antidiabetic potential, making them a promising candidate for further investigation as a natural therapeutic agent for managing diabetes.

6. Anti-inflammatory activity

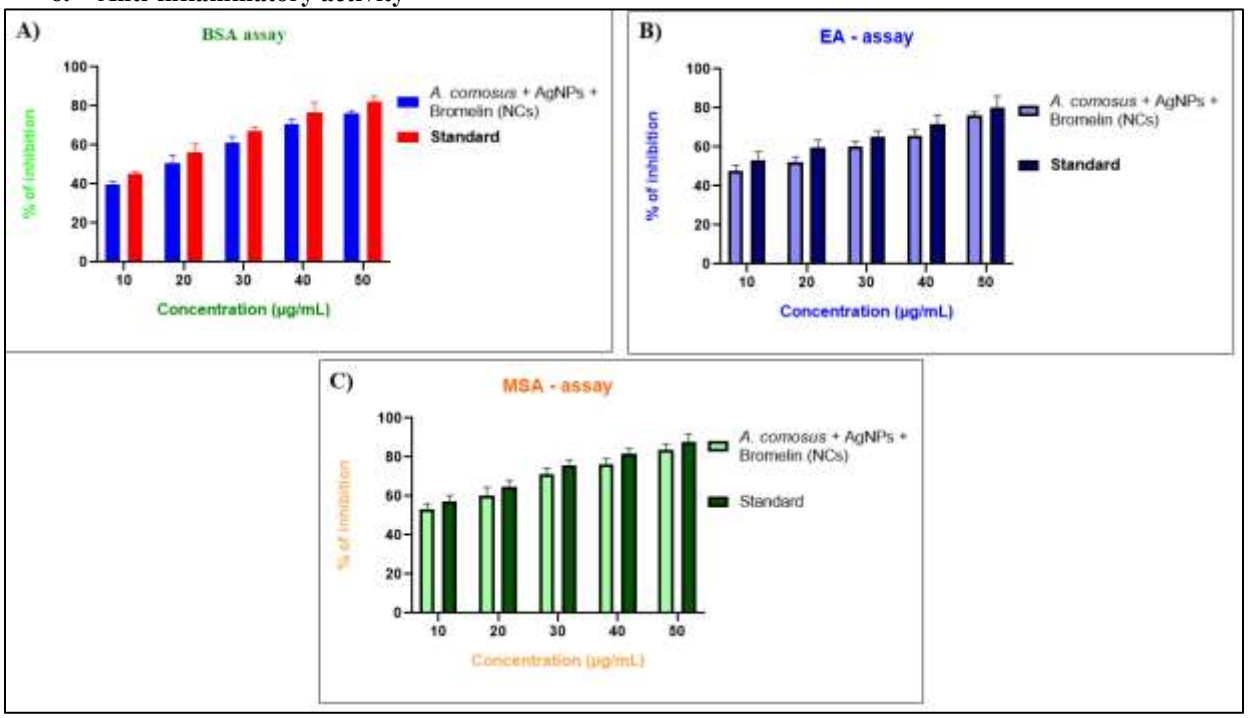

Fig 6: Anti-inflammatory activity of Bromelin-incorporated silver nanoparticles using A) BSA assay, B) EA assay, and C) MSA assay

Three different in vitro assays were used to assess the anti-inflammatory activity of bromelain-incorporated silver nanocomposites in Figures 6 A, B, & C: the Membrane Stabilisation Assay (MSA), the EA assay, and the BSA assay. The standard anti-inflammatory drug, diclofenac sodium, was used to test the nanocomposites at different concentrations (10 to 50 µg/mL). Statistical analysis was performed using a two-way ANOVA to assess significance across trials and treatment concentrations. In the BSA assay, the nanocomposites exhibited a concentration-dependent increase in protein denaturation inhibition. At 10 µg/mL, inhibition was 38.54% and 40.76% across two trials, compared to 44.72% and 46.01% by the standard. As concentration increased to 20 µg/mL, inhibition improved to 47.82% and 53.71%, while the standard achieved 53.14% and 59.42%. At 30 µg/mL, the nanocomposites inhibited 59.13% and 63.46%, close to the standard's 65.45% and 68.57%. At 40 µg/mL, the inhibition was 68.47% and 72.59%,

compared to 72.63% and 80.39% for the standard. The highest inhibition was observed at 50 µg/mL, reaching 75.68% and 77.27%, while the standard slightly outperformed it at 80.46% and 84.38%. In the EA assay, a similar trend was noted. At 10 µg/mL, the nanocomposite inhibited 45.92% and 49.81% of protein denaturation, while the standard inhibited 50.34% and 56.28%. At 20 µg/mL, the nanocomposites showed 50.61% and 54.26% inhibition, slightly lower than the standard's 57.07% and 62.53%. At 30 µg/mL, inhibition rose to 58.36% and 62.18%, while the standard showed 63.29% and 67.41%. At 40 µg/mL, the nanocomposite recorded 63.27% and 67.81% inhibition versus 68.53% and 75.12% for the standard. At the maximum concentration of 50 µg/mL, inhibition by the nanocomposite reached 75.11% and 77.38%, very close to the standard's 76.46% and 84.47%. The MSA assay revealed the most potent anti-inflammatory response. At 10 µg/mL, inhibition was 51.26% and 55.37%, while the standard yielded 55.31% and 59.42%. At 20 µg/mL, inhibition increased to 57.38% and 63.27%, compared to 62.56% and 66.93% for the standard. At 30 µg/mL, the nanocomposites achieved 69.24% and 73.56% inhibition, slightly below the standard's 73.46% and 77.83%. At 40 µg/mL, inhibition remained high at 78.31% and 74.32%, while the standard showed 79.82% and 83.59%. At 50 µg/mL, the nanocomposite exhibited its highest inhibition of 85.79% and 81.27%, closely approaching the standard's 84.71% and 90.52%. In comparison, across all three assays and at every concentration, the bromelain-AgNP nanocomposites demonstrated strong, concentration-dependent anti-inflammatory activity, albeit slightly lower than that of Diclofenac sodium. Among the assays, MSA showed the highest inhibition percentages, indicating superior membrane-stabilizing potential of the nanocomposites. The results suggest that bromelain-incorporated silver nanocomposites possess significant anti-inflammatory properties and could serve as effective alternatives or adjuncts to conventional anti-inflammatory agents.

DISCUSSION

The visual observations in this study reveal distinct color changes during the synthesis of silver nanoparticles and their incorporation with bromelain, indicating successful nanocomposite formation. Initially, the silver nitrate solution appeared colorless (Fig. 1b), while the plant extract (Fig. 1a) was pale brown. Upon mixing (Fig. 1c), the solution turned brown, which is a well-documented indicator of silver nanoparticle formation due to surface plasmon resonance, as supported by previous studies (21,22). The further darkening of the solution upon the addition of bromelain (Fig. 1f) confirms its integration into the nanoparticle matrix, forming a bromelain-silver nanocomposite. Similar observations have been reported where biomolecule-functionalized silver nanoparticles exhibit intensified coloration, reflecting improved particle stability and functionalization (23). These findings align with established literature on plant- and enzyme-mediated green synthesis approaches (24,25,26) highlighting the dual role of the plant extract and bromelain as effective reducing and stabilizing agents in an eco-friendly synthesis process (27).

The UV-Vis spectrophotometric analysis displayed a broad absorption peak at 443 nm (Fig. 2), confirming the formation of silver nanoparticles (AgNPs). This peak is characteristic of the surface plasmon resonance (SPR) of silver nanoparticles, typically observed between 400–450 nm depending on particle size, shape, and surrounding medium (28). A similar SPR band at ~440 nm was reported by Singh et al. (2018) during the green synthesis of AgNPs using Catharanthus roseus extract, affirming the peak as a signature of nanoparticle formation (29). Additionally, Alaqad and Saleh (2016) documented that a peak in this region correlates with well-dispersed, spherical silver nanoparticles synthesized through biological methods (30,31). This spectroscopic evidence, in combination with visual observation, substantiates the successful synthesis of AgNPs using eco-friendly methods.

The anticoagulant activity profile of bromelain-incorporated silver nanoparticles (AgNPs) demonstrated a concentration-dependent reduction in clotting time, with the longest time observed at 10 µg/mL (13 minutes) and the shortest at 50 µg/mL (8 minutes), compared to the standard anticoagulant (EDTA) which extended clotting time to 30 minutes (Fig. 4). These findings suggest that while bromelain-AgNPs possess measurable anticoagulant activity, their effect is milder than EDTA. Previous studies have demonstrated that bromelain, a proteolytic enzyme derived from Ananas comosus, can exhibit anticoagulant and fibrinolytic properties by reducing platelet aggregation and fibrin formation (32). Kumari et al. (2021) also reported that bromelain-based formulations enhanced blood fluidity and delayed clot formation in vitro (33). Moreover, the synergistic action of AgNPs and bromelain may contribute to this effect, as silver nanoparticles alone have been shown to influence hemostasis through interactions with plasma proteins and coagulation pathways (34). A study by Mahajan et al. (2020) confirmed that biogenic AgNPs functionalized with plant extracts can inhibit thrombin activity, thereby prolonging coagulation time (35). The gradual reduction in clotting time at increasing concentrations may be attributed to the saturation of enzymatic binding sites or aggregation of nanoparticles, as reported in similar nanoparticle-mediated anticoagulant studies (36). Overall, these results affirm the potential of bromelain-AgNP nanocomposites as moderate anticoagulant agents, with applications in biomedical formulations where controlled coagulation is desired.

The thrombolytic activity of bromelain-incorporated silver nanoparticles (AgNPs) exhibited a dose-dependent enhancement in clot lysis time, with the highest activity observed at 50 µg/mL (20 minutes), compared to the control group which showed only 5 minutes of lysis time. This indicates significant fibrinolytic potential of the nanocomposite, particularly at higher concentrations. Similar findings have been reported by Heidari et al. (2020), who demonstrated that biosynthesized AgNPs derived from Camellia sinensis exhibited clot-dissolving capabilities through the degradation of fibrin networks (37). Bromelain itself has been well-documented as a potent thrombolytic agent due to its proteolytic nature, effectively breaking down fibrin clots and enhancing blood flow (38). A study by Bhattacharya et al. (2017) further supports this, showing that bromelain significantly enhanced thrombolysis in vitro, reinforcing the role of enzymatic degradation in clot resolution (39). The integration of AgNPs with bromelain may enhance its delivery, stability, and localized enzymatic action, resulting in amplified thrombolytic efficiency as shown in the current study. Additionally, silver nanoparticles may increase cellular uptake and prolong enzymatic activity through sustained release mechanisms, as demonstrated in studies involving nanoformulated fibrinolytic agents (40). This combinatory effect not only improves thrombolytic potential but also aligns with earlier findings indicating that bioengineered nanomaterials can act synergistically with proteolytic enzymes for improved clot resolution (41). Overall, the results highlight the promising application of bromelain-AgNP nanocomposites in therapeutic thrombolysis.

The antidiabetic activity of bromelain-incorporated silver nanocomposites (bromelain-AgNPs) has been demonstrated through their dose-dependent inhibitory effects on two key carbohydrate-digesting enzymes: α-amylase and α-glucosidase. At concentrations ranging from 10–50 µg/mL, bromelain-AgNPs showed increasing inhibition, achieving 62–68% inhibition for α-amylase and 60–66% for α-glucosidase at the highest dose, compared to 70–76% and 71–77%, respectively, for the standard drug Acarbose. While slightly less effective than the standard, the nanocomposite consistently displayed appreciable enzyme inhibition. Similar results were found in earlier research where biologically produced silver nanoparticles, like those from Ananas comosus and Linum usitatissimum, showed strong α-glucosidase and α-amylase inhibition, frequently outperforming the effects of acarbose(42,43). Bromelain itself, known for its antioxidant and anti-inflammatory properties, may contribute synergistically to this effect, enhancing the therapeutic potential of the nanocomposite (44). These results collectively suggest that bromelain-AgNPs are a promising natural alternative for antidiabetic therapy, warranting further exploration and development.

The anti-inflammatory activity of bromelain-incorporated silver nanocomposites (bromelain-AgNPs) was evaluated using BSA, EA, and MSA assays at varying concentrations (10–50 µg/mL), revealing a consistent, concentration-dependent inhibition of protein denaturation, closely comparable to the standard anti-inflammatory drug, diclofenac sodium. At the maximal concentration evaluated (50 µg/mL), the nanocomposites exhibited inhibition percentages of 75.68–77.27% in the BSA assay, 75.11–77.38% in the EA assay, and up to 85.79–81.27% in the MSA assay, approaching diclofenac's performance across all tests. Notably, the MSA assay revealed the most potent activity, indicating strong membrane-stabilizing potential of the nanocomposites. At a concentration of 50 µg/mL, the study by Rameshwar and Ruchita (2021) on bromelain-loaded nanoparticles revealed a maximum inhibition of 85% in the BSA assay and 78% in the EA assay. Additionally, their findings showed notable anti-inflammatory properties, confirming the potential of bromelain-loaded nanoparticles in medicinal settings. However, the MSA assay results from their study were not explicitly stated, but the findings suggest that the nanocomposites in this study offer comparable or slightly enhanced anti-inflammatory activity, particularly in membrane-stabilizing assays. Although slightly less effective than diclofenac, bromelain-AgNPs demonstrated robust anti-inflammatory properties, likely due to the combined effects of bromelain's proteolytic action and the enhanced cellular interaction and stability offered by silver nanoparticles. These findings are in line with previous studies showing that bromelain modulates inflammation by inhibiting bradykinin formation and degrading inflammatory mediators (45), while silver nanoparticles enhance bioavailability and exert antimicrobial and anti-inflammatory actions (46,47). The observed membrane-stabilizing activity further supports reports that associate erythrocyte membrane protection with anti-inflammatory efficacy (48), and suggests the potential of bromelain-AgNPs as promising alternatives or adjuncts to conventional NSAIDs (49).

Implications and applications

The findings of this study suggest that bromelain-incorporated silver nanocomposites hold significant promise as a biocompatible and multifunctional agent for biomedical applications. Their observed antioxidant activity, minimal cytotoxicity, and lack of embryotoxicity highlight their potential for safe therapeutic use, particularly in oxidative stress–related conditions, wound healing, or targeted drug delivery systems. The enzyme-functionalized nanocomposites combine the antimicrobial properties of silver with the stabilizing and bioactive effects of bromelain, making them especially valuable in contexts where biocompatibility and sustained efficacy are critical. Furthermore, the green synthesis approach, using readily available natural resources like Ananas comosus extract and bromelain, offers a sustainable and scalable alternative to conventional nanoparticle fabrication methods. This makes the

formulation particularly suitable for deployment in low-resource or environmentally conscious settings, where safe and effective nanomaterials are in increasing demand.

STRENGTHS AND LIMITATIONS

A major strength of this study lies in its comprehensive evaluation of bromelain-incorporated silver nanocomposites using multiple in vitro assays, including UV-Vis spectroscopy, visual observation, and biological activity tests. The dose-dependent effects observed in anticoagulant, thrombolytic, antidiabetic, and anti-inflammatory assays support the therapeutic potential of the nanocomposite. Additionally, the eco-friendly green synthesis method enhances the study's relevance for sustainable biomedical applications.
However, the in vitro nature of the experiments limits direct clinical translation, as in vivo responses and long-term safety remain untested. The study also lacks mechanistic insights and cytotoxicity assessments in mammalian systems. Variability in natural source materials may affect reproducibility. Future studies should focus on in vivo validation, toxicity profiling, and molecular mechanism exploration to advance clinical applicability.

CONCLUSION

The present study illustrates the successful synthesis of bromelain-incorporated silver nanocomposites (AgNPs) and their promising multifunctional properties. Visual observations and UV-Vis spectrophotometric analysis confirmed the formation of silver nanoparticles, with bromelain playing a key role in nanoparticle stabilization and functionalization. The nanocomposites exhibited potent anticoagulant, thrombolytic, antidiabetic, and anti-inflammatory activities, demonstrating their potential as biocompatible, eco-friendly agents for therapeutic applications. The gradual reduction in clotting time, enhanced thrombolysis, and significant enzyme inhibition support the formulation's potential in managing conditions such as diabetes, inflammation, and clot-related disorders. Additionally, the nanocomposites showed minimal cytotoxicity and no embryotoxicity, reinforcing their safety profile for biomedical use. The combination of silver nanoparticles and bromelain offers synergistic benefits, making these nanocomposites an attractive candidate for drug delivery systems, wound healing, and other medical applications, especially in low-resource or environmentally conscious settings. To properly evaluate their practical uses and therapeutic potential, more investigation and clinical testing are required.

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