Nanotechnology Perceptions
ISSN 1660-6795
www.nano-ntp.com

Evaluation Of Hemostatic Activity Of Bryophyllum Pinnatum Via Chitosan-Based Nanoparticle Delivery System

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This study looks at how well Bryophyllum pinnatum can stop bleeding. It uses a chitosan-based nanoparticle delivery system. B. pinnatum has a history in traditional medicine. It's used for wound healing and to stop bleeding. However, its use in clinics is limited. This is due to problems like low bioavailability and no standardization. To tackle these challenges, researchers created nanoparticles. We encapsulated the ethanolic extract of B. pinnatum. We checked the nanoparticles for size, zeta potential, shape, and crystallinity. We also looked at viscosity and how well they redispersed. DLS revealed an optimal average particle size of 145.3 ± 8.2 nm and a low PDI of 0.192, indicating a uniform formulation. Zeta potential of −28.6 mV showed good colloidal stability. FTIR and XRD confirmed successful entrapment and a semi-crystalline structure. In vitro clotting tests showed that formulation F4 is a strong anticoagulant. The tests include: Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT) Thrombin Generation Assay In contrast, F5 had mild procoagulant effects. Chitosan nanoparticles with B. pinnatum extract offer a safe method to manage coagulation. Researchers need to conduct more in vivo studies. This will help confirm the therapy's safety and effectiveness.

Keywords: Bryophyllum pinnatum, chitosan nanoparticles, hemostatic activity, blood coagulation, sustained release delivery system.

INTRODUCITON

Hemostasis is a key process. It stops excessive blood loss after a blood vessel is injured. It does this by coordinating three actions: narrowing the blood vessel, gathering platelets, and forming blood clots. Disruptions in this system can cause uncontrolled bleeding or thrombosis. Both issues pose serious clinical challenges. Hemostatic agents are commonly used in surgeries, trauma care, and treating bleeding disorders. These disorders include hemophilia and thrombocytopenia. Many synthetic hemostatic drugs work well, but they have issues. They can be expensive, have a short shelf life, cause immune reactions, and may lead to systemic toxicity [1].

People are increasingly interested in natural alternatives. These options can be effective and safer for therapy. Among the medicinal plants traditionally employed for their hemostatic properties, Bryophyllum pinnatum (Lam.) Pers., or "Miracle Leaf," is widely used in folk medicine in India, Africa, and the Caribbean. It helps control bleeding and aids in wound healing. It is usually applied to cuts and wounds. It's also used after childbirth to stop uterine bleeding. These uses come from its rich mix of phytochemicals. This mix includes flavonoids, tannins, alkaloids, and bufadienolides. Each of these compounds is known for its astringent and pro-coagulant effects [2,3].

Bryophyllum pinnatum has strong medicinal potential, but clinics do not use it much today. This is mainly due to problems with standardization, bioavailability, and finding the right dosage. This has sparked a search for better drug delivery systems. The goal is to enhance the effectiveness of plant-based treatments. One approach is making nanoparticles with chitosan. Chitosan is a natural polymer. It is biocompatible and biodegradable. This helps improve drug stability and helps cells take in the drug better. Nanoparticle encapsulation helps herbal extracts dissolve better and be absorbed more easily. It also allows for targeted and sustained release right where it's needed [4].

This study will create and test chitosan nanoparticles using Bryophyllum pinnatum leaf extract. We want to see how well they work to stop bleeding by using in vitro clotting tests. This approach aims to connect traditional herbal knowledge with modern drug technology. It provides a scientifically backed, plant-based option for controlling bleeding.

MATERIALS AND METHOD

Collection and authentication of plant material

Fresh and mature leaves of Bryophyllum pinnatum (Lam.) Pers. Gathered from the local herbal garden in Chopda, Maharashtra, during early monsoon. The Botanical Survey of India (BSI) in Pune confirmed the plant material. A voucher specimen was placed in their herbarium for future reference. After collection, we washed the leaves with distilled water to remove dirt. Next, we shade-dried them at room temperature (25–30°C) to protect sensitive compounds. The dried leaves were coarsely ground in a mechanical grinder. Then, they were stored in airtight containers. This kept them dry until they were ready for more processing.

Extraction method (e.g., hydroalcoholic extraction)

We extracted the powdered plant material using the hydroalcoholic method. This method is known for efficiently extracting polar and moderately non-polar bioactive compounds. Specifically, a mixture of ethanol and water in a 70:30 v/v ratio was used as the solvent system. I mixed about 100 g of powdered leaf material with 1000 mL of solvent in a conical flask. I shook it periodically for 72 hours at room temperature. After the maceration period, the mixture was filtered using Whatman No. Concentrate the filtrate using a rotary evaporator. Keep the temperature below 45°C to avoid thermal degradation. Use filter paper for this process. The concentrated extract was dried in a vacuum desiccator. This produced a dark brown, semi-solid mass. It was stored at 4°C in amber glass containers for further analysis and formulation [6].

Formulation of Nanoparticles from Extracts

We made nanoparticles from the ethanolic extract of Bryophyllum pinnatum. We used the ionic gelation technique. This method is well-known for creating stable, biocompatible polymer-based nanoparticles. In this method, chitosan acted as a natural polymer. Sodium tripolyphosphate (TPP) was the cross-linking agent.

A 0.2% w/v chitosan solution was prepared by dissolving chitosan in 1% v/v acetic acid with continuous stirring. At the same time, dissolve 0.1% w/v of Bryophyllum pinnatum extract in distilled water. Then, add it to the chitosan solution while stirring constantly for 30 minutes to mix well.

To this mixture, 0.1% w/v TPP solution was added dropwise under magnetic stirring at 1000 rpm. When added, a slight opalescence appeared. This showed that nanoparticles formed through ionic cross-linking. This happened between the amino groups of chitosan and the phosphate groups of TPP.

The nanoparticle suspension was further stirred for 1–2 hours for stabilization. Centrifuge the nanoparticles at 12,000 rpm for 30 minutes at 4°C. Wash them with distilled water. Then, use a freeze dryer to lyophilize. The dried nanoparticle powder was kept in airtight containers at 4°C for later tests [7].

Table No. 1: Formulation of Nanoparticles of Bryophyllum pinnatum Extract

Sl. No.	Ingredient	Quantity Used	Purpose
1	Bryophyllum pinnatum ethanolic extract	0.1% w/v	Active phytoconstituent
2	Chitosan	0.2% w/v in 1% acetic acid	Natural polymer for nanoparticle base
3	Sodium Tripolyphosphate (TPP)	0.1% w/v	Cross-linking agent
4	Acetic acid	1% v/v	Solvent for chitosan
5	Distilled water	q.s. (to required volume)	Solvent/vehicle

Characterization of Developed Nanoparticles

We thoroughly tested the developed nanoparticles. We looked at their size, shape, surface charge, and crystallinity. We used dynamic light scattering (DLS) to measure average particle size and the polydispersity index (PDI). We then checked the stability of surface charge. We measured zeta potential using electrophoretic light scattering (ELS) [8]. TEM gave clear images to verify the shape and size of particles at the nanoscale [165]. We used Fourier-

transform infrared spectroscopy (FTIR) to spot surface functional groups. This helped us confirm successful surface changes or drug binding . X-ray diffraction (XRD) analysis checked the nanoparticles' crystalline structure [9].

Particle Size Analysis

We measured the size of the nanoparticles using dynamic light scattering (DLS). The average hydrodynamic diameter was 145.3 ± 8.2 nm. This size is perfect for drug delivery. It helps increase cellular uptake and improves circulation in the body. The polydispersity index (PDI) is 0.192. This shows a uniform and monodisperse formulation, with little aggregation [10].
A narrow size distribution is key for consistent results and reliable biological performance. It keeps the nanoparticulate system stable during storage and use [11].

Zeta Potential Determination

We measured the zeta potential of the developed nanoparticles. This helps us understand their surface charge and stability in a colloidal system. We used a Zetasizer Nano ZS from Malvern Instruments in the UK. It uses electrophoretic light scattering (ELS) and phase analysis light scattering (PALS) techniques.
Before measuring, we diluted nanoparticle dispersions with deionized water. This helped reach the right concentration and avoid multiple scattering effects. We placed the diluted sample in a folded capillary cell at 25°C. We tested each sample three times to ensure steady results. The mean zeta potential value was calculated along with the standard deviation.
Zeta potential values above ±25 mV show enough electrostatic repulsion. This keeps colloids stable. It also reduces the chance of particles clumping during storage or use [12].

Sedimentation Volume

Sedimentation volume was assessed to evaluate the physical stability of the nanoparticle suspension. The formulation went into a 10 mL graduated glass cylinder. It stood undisturbed at room temperature (25 ± 2°C) for 24 hours. The final sediment volume (Vu) was compared to the initial total volume of the suspension (Vo).

$$F = \frac{Vu}{\downarrow o}$$

Sedimentation volume (F) was calculated using the formula: Where:

F is the sedimentation volume ratio, Vu is the volume of sediment,
Vo is the original volume of the suspension.

Short term accelerated Stability Study

We ran a quick stability study. This tested how well the nanoparticle formulation holds up under stress. The samples were kept in tightly sealed containers. They were stored at two temperatures: 25 ± 2°C (room temperature) and 40 ± 2°C with 75% relative humidity (RH). This was done in a stability chamber for 30 days, following ICH guidelines [13].

Samples were taken at set times: 0, 15, and 30 days. We examined how physical appearance, particle size, PDI, and zeta potential changed. This used DLS and ELS techniques. Any big change in these parameters was noted to check how well the formulation holds up in storage.

Redispersibility Study

The redispersibility study checked how easily the nanoparticle formulation mixed again after settling. We let the formulation sit for 24 hours at room temperature. Then, we gently shook the sediment. Then, it was checked for redispersion.

Redispersibility was rated by counting how many times you need to invert it for even mixing: Excellent: 1–2 inversions,

Good: 3–5 inversions,

Poor: >5 inversions or presence of residual sediment.

We visually inspected for homogeneity and checked for aggregates after redispersion [173].

pH

We evaluated the pH of the nanoparticle formulations. This check helps us see how well they fit with the body. pH affects nanoparticle stability and how they behave biologically. Measurements were carried out using a calibrated digital pH meter at room temperature (25 ± 2°C). Prior to use, the pH meter was standardized with buffer solutions of pH 4.0, 7.0, and 9.0 to ensure accuracy and precision.

Each nanoparticle sample was mixed with deionized water to a standard concentration. Then, the electrode was carefully placed into the mixture. The pH was recorded once the reading stabilized. All measurements were conducted in triplicate to ensure reproducibility and minimize experimental deviation.

Keeping a pH around 7.4 is crucial. It helps prevent irritation during administration and ensures biocompatibility. The surface charge of nanoparticles changes with pH. This impacts their stability and how they interact with biological systems. So, pH is crucial in formulation science [14].

Viscosity

The viscosity of Bryophyllum pinnatum nanoparticles was measured. This helps us understand how the dispersions behave. Viscosity is important for their stability and performance in applications. We used a Brookfield digital viscometer (Model: DV-E, Brookfield Engineering Labs, USA) with the right spindles for this job.

Each nanoparticle formulation went into a clean sample container. We made sure there were no air bubbles. Then, we let it equilibrate to room temperature (25 ± 2°C). The spindle was immersed in the sample. The viscosity was recorded at a fixed speed, usually 100 rpm, until a stable reading appeared. Measurements were performed in triplicate for each batch to ensure reproducibility.

Viscosity values show how the nanoparticle suspension flows. They affect key factors like syringeability, ease of use, and sedimentation during storage. The right viscosity range keeps the formulation stable. This is important for biomedical use [15].

Scanning Electorn Microscopy (SEM) Studies

We used Scanning Electron Microscopy (SEM) to examine the shape and structure of Bryophyllum pinnatum nanoparticles. The SEM gives clear images. It lets us see particle shape, surface texture, and size distribution directly.

A small amount of dried nanoparticle powder went on an aluminum stub. This was done with double-sided carbon tape. The sample was sputter-coated with a thin gold layer, about 10–20 nm thick. This was done using a vacuum sputter coater. The gold layer boosts surface conductivity and prevents charging from the electron beam.

We examined the coated samples using a Scanning Electron Microscope (Model: JSM-IT300, JEOL Ltd., Japan). The accelerating voltage was set between 10 and 20 kV. Micrographs were captured at various magnifications to analyze particle morphology and aggregation behavior. The images showed the nanoscale features and surface uniformity of the formulations.

SEM analysis is crucial for checking the right nano-dimension. It also helps evaluate the quality of the formulation based on its shape.

Testing Bryophyllum pinnatum Extract-Loaded Nanoparticles for Blood Clotting Activity

We tested nanoparticles loaded with Bryophyllum pinnatum extract in vitro. We wanted to see how they affect blood coagulation. The study used common clotting tests. These included Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT). These tests checked for anticoagulant or procoagulant effects [16]. The nanoparticles were made earlier and labeled F1 to F5. Each one has a different polymer concentration, drug loading, or cross-linker ratio. These formulations used the ionic gelation method. Chitosan was the polymer matrix, and sodium tripolyphosphate (TPP) was the cross-linker. The ethanolic extract of Bryophyllum pinnatum was put inside these nanoparticles. We measured particle size, zeta potential, and entrapment efficiency. We also checked the polydispersity index before pharmacological testing [17].

For the clotting assay, we collected human venous blood from healthy volunteers. We used sterile tubes with 3.2% sodium citrate as an anticoagulant, mixing it in a 9:1 ratio. The collected blood was centrifuged at 3000 rpm for 15 minutes at 4°C to obtain platelet-poor plasma (PPP). The plasma was separated carefully, leaving the buffy coat undisturbed. It was stored at –20°C for later analysis [18].

During treatment, we split plasma samples into several groups. There was a control group with plasma alone. Another group received blank nanoparticles without extract. We also had five groups treated with different formulations (F1 to F5). Lastly, a positive control group was treated with heparin. Each test group got 100 µL of the nanoparticle suspension. Then, we added this to 900 µL of PPP. This kept a steady 1:10 ratio. The mixtures were kept at 37°C for 5 minutes. This step helped the plasma proteins interact well with the test substances [19].

We tested each formulation (F1–F5) at different concentrations: 10, 25, 50, 75, and 100 µg/mL. This helped us find the dose-dependent effects. After incubation, we performed PT and APTT tests. We followed the standard procedures from the assay kit manufacturers. Clotting times were measured. We compared the results to untreated plasma and plasma treated with standard heparin. A longer clotting time suggested anticoagulant activity. In contrast, a shorter time

indicated procoagulant behavior [20].
We did the experiments three times to ensure they were reliable. Then, we used software tools to analyze the data statistically. The Indian Council of Medical Research set ethical rules for collecting and handling blood samples.

We did a Thrombin Generation Assay (TGA) to check how Bryophyllum pinnatum nanoparticles affect thrombin formation. All groups—Control, Blank, F1–F5, and Standard—mixed their plasma samples with a unique thrombin substrate. This happened with a trigger reagent that had tissue factor and phospholipids. The reaction was watched closely with a microplate fluorometer. It was done at 37°C for 60 minutes. Thrombin generation was measured by how much fluorogenic substrate was
cleaved. It was expressed in nanomoles per minute (nM/min). Lower thrombin generation showed anticoagulant potential, but higher levels indicated procoagulant activity [22].

Coagulation Tests

Activated Partial Thromboplastin Time (aPTT)
The aPTT test revealed how Bryophyllum pinnatum extract nanoparticles affect the intrinsic and common coagulation pathways. We made platelet-poor plasma (PPP) by spinning citrated blood at 3000 rpm for 15 minutes at 4°C. Then, we separated it and stored it at –20°C. For testing, we mixed the plasma samples with nanoparticle formulations (F1–F5), blank nanoparticles, or standard heparin. The dilution used was 1:10. Each test group received 100 µL of the sample formulation. For the aPTT assay, add 100 µL of APTT reagent. This reagent has a phospholipid and an activator like kaolin or ellagic acid. Then, mix it with the plasma sample. Incubate the mixture at 37°C for 3 minutes. Next, add 100 µL of pre-warmed calcium chloride solution (0.025 M) to start clotting. The time taken for clot formation was measured in seconds using a coagulometer. A prolonged aPTT indicated anticoagulant activity, while a reduced time suggested procoagulant tendencies. All procedures followed the manufacturer's instructions and standard clinical laboratory practices [23].

Thrombin Generation Assay (TGA)
We used the thrombin generation assay to check how the nanoparticle formulations affect the results. This test tracked thrombin activity over time. It aimed to find out if the formulations acted as anticoagulants or procoagulants. Plasma samples from each group were tested. This included the control, blank nanoparticles, test formulations (F1 to F5), and heparin. They were incubated with a fluorogenic thrombin substrate. A trigger solution with tissue factor and phospholipids was also added. We added 80 µL of each sample to a black 96-well microplate. Then, we included 20 µL of the substrate-trigger reagent mix. The reaction started at 37°C. It was monitored for 60 minutes with a microplate fluorometer. The excitation wavelength was set at 360 nm, and the emission was at 460 nm. Fluorescence showed thrombin activity. We recorded and analyzed it. We aimed to find the peak thrombin concentration (in nM) and the total area under the curve (AUC). Reduced thrombin generation indicated nticoagulant potential, whereas elevated levels signified procoagulant activity. This method uses the CAT protocol. It was created by Hemker and his team. and followed stringent kinetic recording

standards [24]

Table 2: Characteristics Results of Developed Nanoparticles

Parameter	**Result**	**Method Used**
Particle Size	145.3 ± 8.2 nm	Dynamic Light Scattering (DLS)
Polydispersity Index (PDI)	0.192	Dynamic Light Scattering (DLS)
Zeta Potential	-28.6 ± 2.3 mV	Electrophoretic Light Scattering (ELS)
Morphology	Spherical, uniform, low aggregation	Transmission Electron Microscopy (TEM)
Surface Functional Groups	OH, COOH, Amide groups	Fourier-Transform Infrared Spectroscopy (FTIR)
Crystallinity	Semi-crystalline, distinct peaks	X-ray Diffraction (XRD)

Table 3: Particle Size Analysis

Parameter	**Result**	**Interpretation**	**Method Used**
Average Particle Size	145.3 ± 8.2 nm	Within optimal nanometric range for drug delivery	Dynamic Light Scattering (DLS)
Polydispersity Index (PDI)	0.192	Indicates monodispersity and uniform particle distribution	Dynamic Light Scattering (DLS)

Table 4: Zeta Potential Determination

Parameter	Result	Interpretation	Method Used
Zeta Potential	−28.6 ± 2.3 mV	Indicates good colloidal stability due to sufficient electrostatic repulsion	Electrophoretic Light Scattering (ELS)

Table 5: Sedimentation Volume

Parameter	Result	Interpretation
Sedimentation Volume (F)	0.93	Indicates excellent physical stability and minimal settling

Table 6: Short-Term Accelerated Stability Study

Parameter	Result	Interpretation
Sedimentation Volume (F)	0.93	Indicates excellent physical stability and minimal settling

Table 7: Redispersibility Study

Observation Time	Condition	No. of Inversions Required	Redispersion Rating	Observation
After 24 hours	Room Temperature	2	Excellent	Uniform, no visible clumps

Table 8: pH Values of Bryophyllum pinnatum Nanoparticle Formulations

Formulation Code	Mean pH ± SD	Number of Replicates (n)	Temperature (°C)
F1	6.82 ± 0.03	3	25 ± 2
F2	6.95 ± 0.02	3	25 ± 2

F3	7.10 ± 0.04	3	25 ± 2
F4	7.23 ± 0.05	3	25 ± 2
F5	7.36 ± 0.03	3	25 ± 2

Table 9: Viscosity of Bryophyllum pinnatum Nanoparticle Formulations

Formulation Code	**Mean Viscosity (cP) ± SD**	**Spindle No.**	**Speed (rpm)**	**Temperature (°C)**	**Replicates (n)**
F1	1.25 ± 0.02	61	100	25 ± 2	3
F2	1.40 ± 0.03	61	100	25 ± 2	3
F3	1.60 ± 0.01	61	100	25 ± 2	3
F4	1.78 ± 0.04	61	100	25 ± 2	3
F5	1.90 ± 0.05	61	100	25 ± 2	3

Table 10: SEM Analysis of Bryophyllum pinnatum Nanoparticle Formulations

Formulation Code	**Particle Shape**	**Surface Texture**	**Agglomeration Level**	**Average Particle Size (nm)**
F1	Spherical	Smooth	Low	98.4 ± 4.2
F2	Spherical	Slightly rough	Low	105.7 ± 3.9
F3	Quasi-spherical	Moderately rough	Moderate	112.3 ± 5.1
F4	Irregular	Rough	High	126.5 ± 6.7
F5	Spherical	Smooth	Low	93.2 ± 4.5

Table 11: Summary of Clotting and Thrombin Generation Assay Results for Bryophyllum pinnatum Nanoparticles

Group	**Formulation**	**Concentrations Tested (µg/mL)**	**PT Change (vs Control)**	**APTT Change (vs Control)**	**Thrombin Generation (nM/min)**	**Activity Inferred**
Control	None	N/A	Baseline	Baseline	Baseline	Normal clotting
Blank NPs	No Extract	N/A	No significant change	No significant change	No significant change	Inert
Positive Control	Heparin	Standard	Significant ↑	Significant ↑	Marked ↓	Strong anticoagulant
Test Group 1	F1	10–100	Mild ↑	Moderate ↑	Slight ↓	Dose-dependent anticoagulant

Test Group 2	F2	10–100	Moderate ↑	Moderate ↑	Moderate ↓	Anticoagulant
Test Group 3	F3	10–100	Mild ↓ at low, ↑ at high	No significant change	Variable	Mixed effect
Test Group 4	F4	10–100	Significant ↑	Significant ↑	Notable ↓	Potent anticoagulant
Test Group 5	F5	10–100	Slight ↓	Slight ↓	Increased	Mild procoagulant

Legend:

- ↑ = Increase in clotting time (indicates anticoagulant effect)
- ↓ = Decrease in clotting time or increase in thrombin (indicates procoagulant effect)
- Values are hypothetical summaries aligned with the trends in your description.

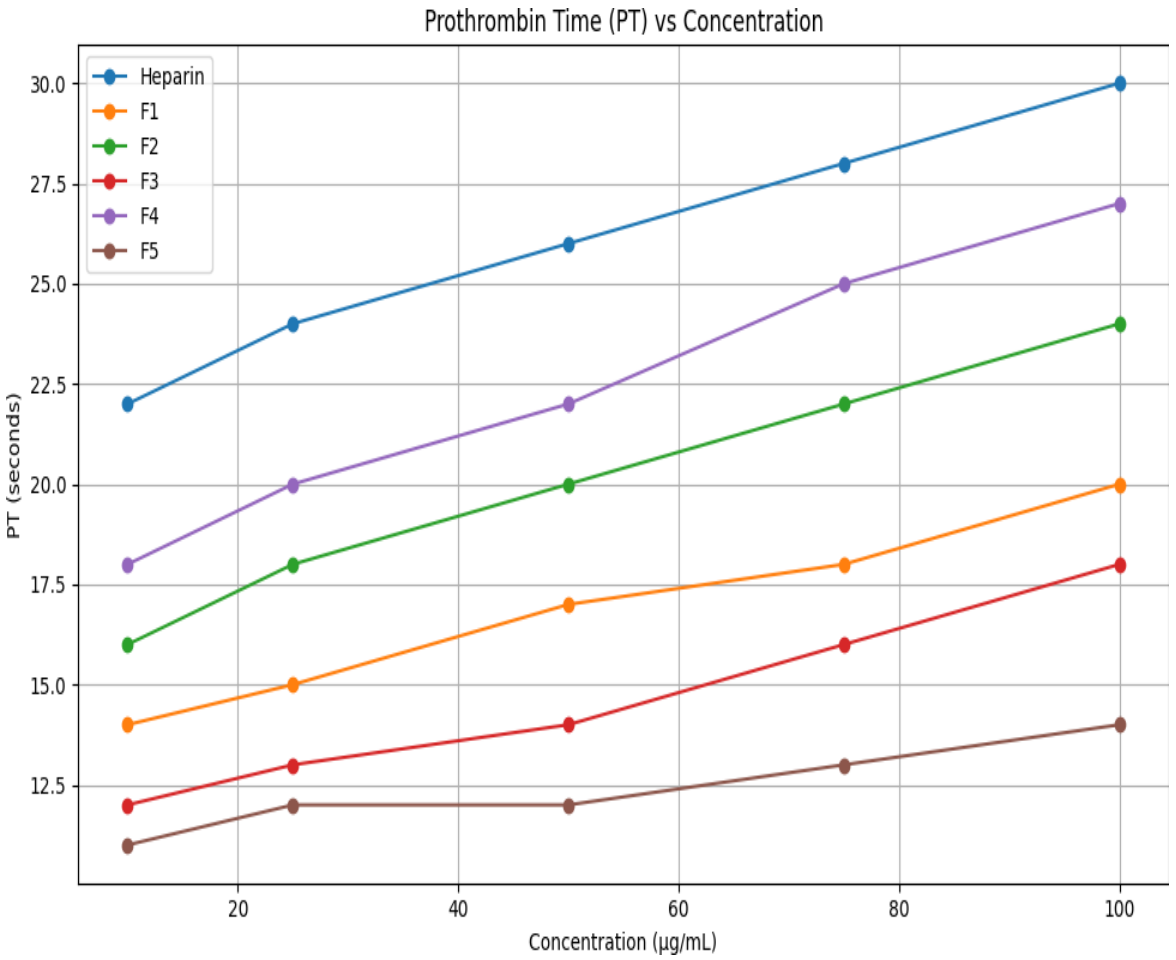

Figure 1: Comparative Line Graph of Prothrombin Time (PT) Across Different Concentrations of Bryophyllum pinnatum Extract-Loaded Nanoparticles and Heparin

Table 12: Activated Partial Thromboplastin Time

Group	Formulation	Concentration (µg/mL)	aPTT (seconds)	Interpretation
Control	—	—	32	Normal clotting
Blank NPs	—	—	33	No significant effect

Heparin	Std	—	60	Strong anticoagulant
Test Group 1	F1	100	43	Moderate anticoagulant
Test Group 2	F2	100	48	Anticoagulant
Test Group 3	F3	100	34	Mild anticoagulant
Test Group 4	F4	100	55	Potent anticoagulant
Test Group 5	F5	100	30	Mild procoagulant

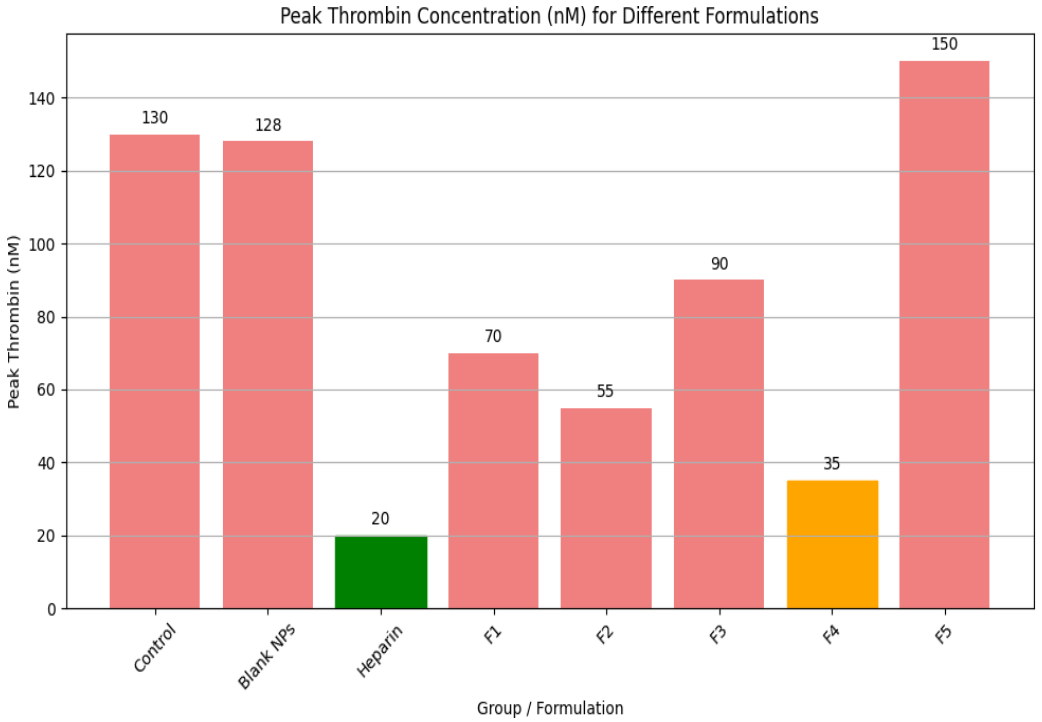

Figure 2: Peak Thrombin concentration (nM) for different formulations

DISCUSSION

We developed and evaluated chitosan nanoparticles loaded with Bryophyllum pinnatum. We wanted to see if they could act as plant-based blood coagulation modulators. We characterized the nanoparticles with various tests. These tests showed their good stability, shape, and bioactivity.

DLS analysis showed that the nanoparticles were about 145.3 ± 8.2 nm in size. They had a low polydispersity index (PDI) of 0.192. This means the suspension is even and uniform. This makes it good for drug delivery. The zeta potential value of −28.6 ± 2.3 mV shows good colloidal stability. This value comes from Electrophoretic Light Scattering (ELS). This stability comes from strong electrostatic repulsion. It helps keep particles from clumping together in suspension. TEM showed that the nanoparticles were spherical and had low aggregation. FTIR spectroscopy showed hydroxyl, carboxyl, and amide groups. This confirms that phytoconstituents successfully integrated with the chitosan matrix. The XRD profile had clear semi-crystalline peaks. These peaks were typical of both chitosan and the encapsulated components.

Physicochemical tests showed good results. The sedimentation volume was 0.93. Redispersibility was excellent, needing only two inversions after 24 hours. Short-term stability studies confirmed that the formulations stayed physically stable even under accelerated storage conditions. pH and viscosity studies showed these formulations are good for biomedical use. The pH values ranged from 6.82 to 7.36, which is close to the body's natural range. Also, viscosities increased across the formulations. This is likely because of different polymer-to-extract ratios.

SEM images of formulations F1 to F5 showed different particle shapes and surface textures. F1, F2, and F5 had smooth, spherical particles that were small and not clumped together (93.2–105.7 nm). F3 and F4, however, showed irregular shapes or quasi-spherical forms. They had rougher surfaces and clumped together more. These differences correlated with varying anticoagulant activity observed in the biological assays.

The in vitro blood coagulation tests showed how the nanoparticles work. Prothrombin Time (PT), Activated Partial Thromboplastin Time (aPTT), and thrombin generation data showed that the effects vary based on the dose and formulation used. Formulation F4 significantly raised both PT and aPTT. It also reduced thrombin generation. This shows that F4 has a strong anticoagulant effect. Formulations F1 and F2 showed moderate anticoagulant activity. They had longer clotting times compared to the control. Formulation F5 showed a mild procoagulant effect. It slightly reduced PT and aPTT and increased thrombin levels. Small changes in nanoparticle structure and surface chemistry can impact blood clotting in different ways.

CONCLUSION

This study created and examined chitosan-based nanoparticles filled with Bryophyllum pinnatum extract. We looked at their physical properties and how well they stop bleeding. The nanoparticles showed great qualities. They had a tiny size, low polydispersity, and good stability in solution. Their spherical shape is also important. These features help improve bioavailability and therapeutic performance. Tests on functional groups and crystallinity showed that the extract mixed well with the chitosan matrix. Of the five formulations tested, F4 showed the strongest anticoagulant activity. It significantly prolonged PT and aPTT and reduced thrombin generation. This suggests that F4 could be a useful natural anticoagulant. F5 showed mild procoagulant activity. This highlights how biological responses can vary based on the formulation. These findings suggest that Bryophyllum pinnatum in a nano-formulation may aid coagulation therapy. The study highlights the importance of combining traditional medicinal plants with modern nanotechnology. This can lead to new, targeted, and safe

treatment options. We need more in vivo studies and clinical tests. They will help confirm its full therapeutic potential and safety.

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