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In-Vitro Anticoagulant and Thrombolytic Properties of Crude *Aspergillus terreus* Extract

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Abstract

Thrombosis remains a major global health concern, contributing to cardiovascular disease, stroke, and venous thromboembolism. Current pharmacologic agents, such as heparin and streptokinase, act either as anticoagulants or thrombolytics but rarely combine both mechanisms. Natural products, particularly fungal metabolites and enzymes, are increasingly explored as alternative or complementary sources of bioactive compounds. This study evaluated the anticoagulant and thrombolytic potential of crude Aspergillus terreus extract in vitro. An experimental, laboratory-based design was employed. Whole blood clotting time, prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT) were measured in plasma from healthy donors treated with graded concentrations of A. terreus extract. Thrombolytic activity was assessed by weight-based clot lysis assays, with unfractionated heparin serving as the anticoagulant control, streptokinase as the thrombolytic control, and phosphate-buffered saline as the vehicle control. Data were analyzed using ANOVA with post hoc Scheffé testing, with significance set at $p < 0.05$. The extract significantly prolonged clotting times in a dose-dependent manner. In whole blood assays, clotting was extended beyond normal ranges, with effect sizes classified as large. PT values increased to over 30 seconds, plateauing between 20 and 30 mg/mL, while aPTT rose sharply at 30 mg/mL, exceeding 80 seconds, suggesting preferential effects on the intrinsic pathway. Heparin consistently produced the longest clotting times. In thrombolysis assays, the extract achieved up to 45% clot lysis, significantly greater than heparin and saline controls, and not statistically different from streptokinase despite lower absolute means. Aspergillus terreus crude extract exhibits dual antithrombotic activity, combining anticoagulant and thrombolytic effects. While less potent than heparin or streptokinase alone, its ability to influence both clot formation and clot dissolution highlights its novelty as a natural therapeutic source. Future research should isolate and characterize active constituents, expand replication for statistical robustness, conduct safety and cytotoxicity profiling, and validate efficacy in vivo to determine translational potential.

Keywords— Aspergillus terreus, anticoagulant activity, thrombolytic activity, prothrombin time, activated partial thromboplastin time, clot lysis assay, natural products, fungal biotechnology

1. Introduction

Thrombosis and its clinical consequences remain among the leading causes of global mortality and morbidity. Atherosclerotic cardiovascular disease, ischemic stroke, and venous thromboembolism (VTE) together account for a substantial share of deaths and disability-adjusted life years, with estimates suggesting that thrombosis contributes to roughly one in four deaths worldwide (Raskob & Wendelboe, 2016; Wendelboe & Raskob, 2016). Pathophysiologically, thrombus formation reflects a dysregulated hemostatic response in which platelet activation, coagulation cascade amplification, and fibrin polymerization proceed unchecked, producing occlusive clots that impair tissue perfusion and precipitate end-organ injury (Swieringa et al., 2025).

While the hemostatic system is indispensable for limiting hemorrhage after vascular injury, its misdirection underlies a broad spectrum of arterial and venous events that demand both prophylactic and therapeutic anticoagulation.

For decades, unfractionated heparin (UFH) and its derivatives have formed the backbone of antithrombotic care. Heparin potentiates antithrombin to inhibit thrombin (factor IIa) and factor Xa, thereby reducing fibrin formation and preventing clot propagation (Banik et al., 2021; Oduah et al., 2016). Clinically, UFH is invaluable for rapid anticoagulation in acute coronary syndromes, pulmonary embolism, and peri-procedural settings; it is typically monitored with activated partial thromboplastin time (aPTT), while prothrombin time (PT) is more often used for vitamin-K antagonists but may be prolonged at high heparin concentrations (Mijovski, 2019; Alexander et al., 2021).

Despite its centrality, heparin therapy carries nontrivial limitations: dose variability, need for laboratory monitoring, bleeding complications, and the risk of heparin-induced thrombocytopenia (HIT)—an immune-mediated prothrombotic syndrome with limb- and life-threatening potential (Pollak, 2019). Long-term UFH exposure has also been associated with osteopenia/osteoporosis and abnormal liver enzymes (Douketis, 2022). Beyond safety, supply-chain vulnerabilities—most visibly the 2007–2008 contamination crisis—highlight the fragility of heparin's raw-material pipeline and the need for diversified, safer anticoagulant sources (Zhu et al., 2019).

Concurrently, interest has grown in bioactive compounds from plants and fungi that modulate coagulation and fibrinolysis. Fungal metabolites and enzymes have long seeded pharmacotherapy (e.g., statins from Aspergillus terreus), and accumulating evidence suggests that fungal polysaccharides and proteases can exert anticoagulant and antithrombotic effects in vitro and in vivo (Bian et al., 2020; Subhan et al., 2016; Amer et al., 2020). Of particular mechanistic relevance is collagen's role in primary and secondary hemostasis: upon vascular injury, exposed collagen serves as a potent substrate for platelet adhesion and activation and contributes to thrombus stability through interactions with von Willebrand factor and integrins (Manon-Jensen et al., 2016; Farndale et al., 2016).

Enzymes capable of degrading collagen—collagenases—could, in principle, attenuate collagen-mediated platelet activation or destabilize the extracellular matrix contributions to thrombus formation, complementing anticoagulant effects on the cascade and, potentially, enhancing clot dissolution under certain experimental conditions.

Aspergillus terreus, a filamentous fungus ubiquitous in soil and industrial biotechnology, produces a diverse array of secondary metabolites (e.g., lovastatin, terrein) and enzymes, including collagenolytic proteases (Costa-Junior et al., 2019; Vassileva et al., 2020; Asfour et al., 2019). Collagenase activity from Aspergillus spp. has been documented and, in some settings, linked to changes in coagulation metrics and prothrombin time (Ferreira et al., 2016; Wanderley et al., 2017; Costa-Junior et al., 2019).

More broadly, serine protease inhibitors (SERPINs) and coumarin-like metabolites—found across fungal taxa—intersect key nodes of coagulation and fibrinolysis, underscoring fungi as credible sources of antithrombotic leads (Bianchini et al., 2021; Breidenbach et al., 2020; Cheke et al., 2022). Against this backdrop, identifying, characterizing, and benchmarking fungal preparations with anticoagulant and/or thrombolytic potential against clinical standards is a pragmatic research pathway.

The present work is motivated by two converging needs: (1) safer, scalable, and potentially cost-effective antithrombotic options that do not inherit heparin's immunogenic or supply-chain liabilities; and (2) mechanistic exploration of fungal enzyme systems that may influence both coagulation and clot stability. Specifically, we focus on a crude extract of A. terreus, hypothesizing that its proteolytic profile (including collagenase) may prolong coagulation times and demonstrate in-vitro clot lysis activity—effects that, if confirmed, could justify downstream fractionation and target isolation.

2. Review of Related Literature

2.1 Hemostasis, thrombosis, and current anticoagulant strategies

Hemostasis involves tightly regulated platelet plug formation (primary hemostasis) and fibrin generation through intrinsic, extrinsic, and common cascade pathways (secondary hemostasis) (Swieringa et al., 2025). Dysregulation yields thrombosis, in which intravascular clots obstruct arterial or venous flow, contributing to myocardial infarction, ischemic stroke, and VTE (Raskob & Wendelboe, 2016; Othieno et al., 2018). Anticoagulants interrupt discrete coagulation steps: UFH/LMWHs potentiate antithrombin against thrombin/Xa; vitamin-K antagonists reduce synthesis of vitamin-K–dependent factors; and newer direct oral anticoagulants target thrombin or factor Xa (Mekaj et al., 2015; Kim et al., 2017). Monitoring differs by agent: UFH is conventionally tracked with aPTT, while PT/INR guides warfarin therapy (McRae, et al. 2021).

Limitations of heparin-based care

UFH's dose–response variability necessitates laboratory monitoring and titration; bleeding is the principal clinical hazard. HIT remains a critical

safety concern, with paradoxical thrombotic risk due to anti-PF4/heparin antibodies (Pollak, 2019). Longer-term adverse effects, including bone mineral loss, have also been reported (Douketis, 2022). Additionally, the heparin contamination episode exposed vulnerabilities in global supply and quality controls for naturally derived, structurally heterogeneous glycosaminoglycans (Zhu et al., 2019; Hao et al., 2019; Banik et al., 2021).

2.2 Fungal sources of anticoagulant and thrombolytic activity

Natural products pipelines increasingly mine fungi for antithrombotic candidates. Polysaccharides from edible and medicinal fungi demonstrate anticoagulant activity in vitro, sometimes via interactions with thrombin or factor Xa (Bian et al., 2020). Enzymatic systems from Aspergillus spp. can show anticoagulant, fibrinolytic, or plasma-coagulant effects depending on substrate specificity and preparation (Osmolovskiy et al., 2021). A. terreus, in particular, is biotechnologically prominent for statin biosynthesis and other bioactives (Subhan et al., 2016; Vassileva et al., 2020; Amer et al., 2020).

Evidence for A. terreus–derived collagenase includes production using proteinaceous substrates and ultrasound-assisted hydrolysis yielding bioactive peptides (Ferreira et al., 2016; Costa-Junior et al., 2019). Conceptually, by degrading collagen—a key ligand for platelet adhesion/activation—collagenases might blunt primary hemostasis, while broader proteolytic activity could (under certain assay conditions) facilitate partial dissolution of pre-formed clots, although classical fibrinolysis is mediated by plasmin rather than collagenase (Manon-Jensen et al., 2016; Farndale et al., 2016).

2.3 Assay platforms to characterize antithrombotic effects

In-vitro coagulation assays (TT, PT, aPTT) provide orthogonal readouts of cascade interference. TT reports conversion of fibrinogen to fibrin; PT reflects extrinsic/common pathways, and aPTT reflects intrinsic/common pathways—each prolonged by different classes of inhibitors (Winter et al, 2017). In-vitro thrombolysis models, which quantify percent clot lysis after adding a candidate agent to a pre-formed clot, offer a pragmatic screen and typically include streptokinase as a positive control (Zaman et al., 2015; Muduli et al., 2022; Frühwald et al., 2019). Heparin, notably, lacks fibrinolytic activity and thus is not expected to lyse formed clots in such assays; its contribution is to prevent extension and propagation (Facep, 2021; Alexander et al., 2021).

2.4 Buffer systems and experimental controls

Phosphate-buffered saline (PBS) offers an isotonic, near-physiologic environment for ex vivo blood studies and is generally non-perturbing to red-cell morphology, making it a suitable vehicle/control (Gupta et al., 2016; Son et al., 2021; AAT Bioquest, 2019). Dose–response designs using graded extract concentrations enable preliminary potency and selectivity insights across PT/aPTT/TT panels and thrombolysis readouts.

2.5 Gaps in the Literature

Despite promising signals, several substantive gaps hinder translation:

1. Species- and preparation-specific evidence is sparse. Many reports aggregate across fungal genera or emphasize polysaccharides, leaving enzyme-rich crude extracts from specific species—such as A. terreus—under-characterized with respect to anticoagulant and thrombolytic potential. There is limited head-to-head comparison of A. terreus extracts with standard-of-care anticoagulants across a uniform assay battery (PT, aPTT, TT, and quantitative thrombolysis).
2. Benchmarking against clinical standards is limited. Few studies situate fungal preparations against UFH in the same experimental system. Given heparin's non-fibrinolytic profile, incorporating both cascade assays (where heparin is active) and thrombolysis (where streptokinase is active) is essential to contextualize what a fungal extract can—and cannot—do relative to the clinical toolkit.
3. Mechanistic resolution is preliminary. Crude extracts contain a mélange of proteases, inhibitors, and secondary metabolites. Without pathway-selective assays (PT vs aPTT vs TT), inhibitor controls, or subsequent fractionation, it remains unclear whether observed effects stem from collagenase-mediated attenuation of platelet/collagen interactions, direct interference with cascade proteases, nonspecific proteolysis of fibrin/fibrinogen, or ancillary metabolites (e.g., coumarin analogues) (Wanderley et al., 2017; Bianchini et al., 2021; Breidenbach et al., 2020).
4. Dose–response and formulation optimization are underreported. Systematic evaluation of extract concentration and extract:buffer ratios (e.g., with PBS) across multiple readouts is uncommon, yet essential for estimating potency, therapeutic window, and practical formulation parameters for downstream development.
5. Safety and translational considerations remain to be mapped. In vitro anticoagulant and thrombolytic activity do not alone establish

safety. While collagenase has been used clinically in other indications without prohibitive bleeding risks under specific conditions (Amighi et al., 2020), A. terreus extracts warrant careful toxicologic and immunogenicity profiling, including potential off-target proteolysis.

6. Quality and supply chain standardization are needed. Lessons from heparin underscore the importance of traceable, standardized production for any naturally derived anticoagulant candidate (Zhu et al., 2019). Fungal biomass cultivation, extraction solvents, and process parameters can all affect composition and activity; documenting these with rigor is a precondition for reproducibility.
7. Addressing these gaps requires a staged strategy: (i) a robust, comparative in-vitro characterization of A. terreus crude extract against UFH and appropriate positive/negative controls; (ii) identification of pathway selectivity via PT/aPTT/TT; (iii) quantification of in-vitro clot lysis relative to streptokinase; and (iv) determination of an initial "optimal" extract concentration/ratio for maximal effect with acceptable assay behavior—thus laying the empirical basis for subsequent fractionation and mechanistic work.

2.6 Objectives

Primary Objective

To compare the anticoagulant activity of Aspergillus terreus crude extract with unfractionated heparin in vitro, as measured by standard coagulation assays—prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT)—under controlled laboratory conditions.

Secondary Objectives

- To evaluate the in-vitro thrombolytic effect of A. terreus crude extract on pre-formed clots and benchmark its lytic activity against a positive control (streptokinase) and a negative control (vehicle), recognizing that heparin is not a thrombolytic agent.
- To characterize the concentration–response profile of the A. terreus crude extract and identify an optimal extract:buffer (PBS) ratio that maximizes anticoagulant and/or thrombolytic readouts without producing assay artifacts.
- To infer preliminary pathway selectivity by comparing the relative magnitude of PT, aPTT, and TT prolongation, thereby generating hypotheses about potential targets (e.g., intrinsic vs extrinsic/common pathways; fibrinogen conversion).
- To establish an empirical foundation for future mechanistic and translational work, including planned fractionation of the crude extract, identification of active moieties (e.g., collagenase or other proteases/inhibitors), and safety-relevant assessments in subsequent studies.

3. Methodology

3.1. Research Design

This investigation adopted an experimental, in-vitro comparative design to determine the anticoagulant and thrombolytic activity of a crude extract obtained from Aspergillus terreus. The approach was chosen because in-vitro assays provide a controlled and reproducible environment to establish initial proof-of-concept evidence without exposing human or animal subjects to unnecessary risk. The study compared the performance of the fungal extract with established pharmacological standards, particularly unfractionated heparin, a reference anticoagulant, and streptokinase, a reference thrombolytic.

In addition, phosphate-buffered saline (PBS) was used as a neutral vehicle to serve as the negative control. The design permitted systematic evaluation of clotting times and clot dissolution across different treatment categories and concentrations, ensuring that any observed effects could be attributed to the test extract rather than to background variability.

3.2 Samples and materials

The biological material used in this study consisted of human blood samples collected from a pool of twenty healthy adult donors, both male and female, aged between 20 and 40 years. Individuals were included only if they were non-smokers, had no history of hematological disorders, and were free from medications that could interfere with coagulation, such as anticoagulants, antiplatelets, or non-steroidal anti-inflammatory drugs.

3.3 Methods and Procedures

Exclusion criteria encompassed conditions such as pregnancy, liver disease, anemia, recent infection, or any other clinical history that could distort coagulation profiles. Prior to donation, each participant was informed of the nature and purpose of the study and signed an informed consent form. Venipuncture was performed under aseptic conditions using sterile disposable syringes, and blood was collected into tri-sodium citrate tubes containing 3.2% anticoagulant to prevent clotting during handling. Plasma was separated by

centrifugation at 3,000 revolutions per minute for 15 minutes at room temperature. The supernatant was carefully transferred to sterile containers and maintained on ice until assay, which was carried out within four hours of collection to preserve sample integrity.

The preparation of the fungal extract followed standard microbiological and biochemical procedures. Soil samples were first collected under sterile conditions, and A. terreus was isolated and identified on the basis of its characteristic morphology, including colony pigmentation and conidial structures. Although molecular confirmation using ITS sequencing remains the gold standard, for the present exploratory work, morphological features were considered sufficient for accurate identification.

The isolate was cultured in Sabouraud dextrose broth and incubated at 28 °C for seven days under constant agitation at 150 revolutions per minute to encourage secretion of extracellular metabolites. At the end of the incubation period, the culture was filtered through muslin cloth to separate the mycelial biomass from the extracellular broth.

To concentrate the proteinaceous fraction, ammonium sulfate precipitation was performed at 80% saturation, followed by dialysis against phosphate-buffered saline (pH 7.4, 0.01 M) in order to remove residual salts. The resulting crude extract was lyophilized and subsequently reconstituted in PBS to obtain the working stock solution. The protein content of the preparation was standardized using the Bradford assay to ensure consistency across experimental replicates.

3.4 Coagulation Assays

For the anticoagulant evaluation, three standard coagulation assays were employed: prothrombin time (PT), activated partial thromboplastin time (aPTT), and thrombin time (TT). Each of these assays targets different portions of the coagulation cascade, thus allowing preliminary inferences regarding the pathway selectivity of the test extract.

Prothrombin time was determined by incubating citrated plasma with the fungal extract and subsequently initiating coagulation with thromboplastin reagent containing tissue factor and calcium. Activated partial thromboplastin time was measured by preincubating plasma with cephaloplastin reagent and the extract, followed by the addition of calcium chloride to trigger clot formation.

Thrombin time was obtained by adding exogenous thrombin to plasma pretreated with the extract and measuring the interval required for fibrin clot development. In all cases, the samples were maintained at 37 °C to approximate physiological conditions, and clotting times were recorded using a semi-automated coagulometer. Normal baseline ranges were established from pooled control plasma, with PT between 12 and 15 seconds, aPTT between 28 and 40 seconds, and TT between 15 and 20 seconds. Prolongation beyond these ranges was interpreted as indicative of anticoagulant activity.

In addition to coagulation testing, an in-vitro thrombolysis assay was conducted to evaluate whether the fungal extract possessed the ability to lyse pre-formed clots. This procedure involved clot formation by incubating whole blood with calcium chloride until complete coagulation occurred. After serum removal, the clots were carefully weighed to establish the initial mass.

Each clot was then treated with the test solution, incubated at 37 °C for 90 minutes, and reweighed after the supernatant was discarded. The extent of clot dissolution was calculated as the percentage reduction in clot mass relative to the initial weight. Streptokinase served as the positive thrombolytic control, demonstrating expected lytic activity, while PBS functioned as the negative control.

Heparin was also included in the assay to confirm its lack of thrombolytic activity, since its clinical role is confined to prevention of clot extension rather than dissolution. All experiments were performed in triplicate for each donor to minimize random error and account for biological variability.

3.5 Data Collection and Analysis

Data collection involved recording clotting times in seconds for PT, aPTT, and TT, and computing the mean percentage clot lysis for the thrombolysis assay.

All values were expressed as mean ± standard deviation. Statistical analysis was carried out using one-way analysis of variance (ANOVA) to test for overall group differences, followed by Tukey's honestly significant difference (HSD) test to determine pairwise differences among treatments. Linear regression was also applied to explore concentration–response relationships between fungal extract levels and clotting time prolongation or thrombolysis. A significance threshold of $p < 0.05$ was adopted throughout the analyses. All statistical procedures were conducted using SPSS version 25.

3.6 Ethical considerations

Ethical considerations were rigorously observed. The study was reviewed and approved by the Institutional Ethics Review Committee of Centro Escolar University, and all procedures conformed to the principles outlined in the 2013 revision of the Declaration of Helsinki. Volunteer donors were

informed about the purpose, risks, and benefits of participation, and written consent was obtained prior to blood collection. Anonymity and confidentiality were preserved by coding donor samples and ensuring that no personally identifiable information was linked to experimental data.

The methodological choices in this research were grounded in both scientific rationale and practical constraints. Employing crude extracts allowed the preservation of synergistic interactions among the diverse metabolites produced by A. terreus, a strategy that is typical of initial pharmacological screenings. The use of PT, aPTT, and TT assays provided a comprehensive assessment of potential effects across the intrinsic, extrinsic, and common pathways of coagulation.

The inclusion of heparin and streptokinase as controls anchored the study's findings within clinically relevant benchmarks, distinguishing between anticoagulant and thrombolytic profiles. The selection of PBS as a vehicle was based on its physiological compatibility and minimal effect on red cell morphology. Triplicate testing across multiple donor samples further strengthened the reliability of the findings by accounting for both technical and biological variability.

Nonetheless, several limitations of the methodology are acknowledged. The crude nature of the extract prevents precise identification of the active constituents responsible for any observed anticoagulant or thrombolytic effects. In-vitro assays, while valuable for screening, cannot fully replicate the complexity of in-vivo hemodynamic conditions, endothelial interactions, or metabolic clearance. The exclusive use of blood from healthy donors also means that the results may not directly translate to patient populations with coagulopathies or prothrombotic disorders. Furthermore, cytotoxicity and hemolysis assays were not incorporated at this stage, although these will be essential components of subsequent safety evaluations.

Overall, this methodology provides a robust experimental framework for the preliminary assessment of Aspergillus terreus crude extract as a potential source of anticoagulant or thrombolytic agents. It balances reproducibility with clinical relevance by combining standardized coagulation assays, an established thrombolysis model, and comparative controls. The results derived from this design are expected to inform not only the biological plausibility of fungal extracts as anticoagulant candidates but also the direction of future research, which will include fractionation, mechanistic characterization, and eventual translational studies in vivo.

4. Results and Discussion

4.1 Results

The results of this study are presented in sequence according to the experimental assays performed. For clarity, each table is inserted at the relevant point, with interpretation and discussion of the observed trends.

Table 1: Measurements of the Blood Clotting Time

Set up	Trial 1	Trial 2	Trial 3	Average Clotting Time
10 mg/mL *A. terreus*	10 mins 30 secs	15 mins 30 secs	14 mins 30 secs	13 mins 30 seccs
20 mg/mL *A. terreus*	13 mins 30 secs	16 mins 30 secs	15 mins 30 secs	15 mins
30 mg/mL *A. terreus*	15 mins 30 secs	20 mins 30 secs	19 mins 30 secs	18 mins 30 secs
Heparin	-	-	-	-
Control (NSS)	5 mins 30 secs	6 mins 30 secs	11 mins 30 secs	8 mins

Table 1 presents the blood clotting times of samples treated with different concentrations of Aspergillus terreus extract, with heparin, and with saline solution as the control. The data reveal that increasing concentrations of the fungal extract were associated with longer clotting times. Specifically, the 30 mg/mL extract prolonged clotting to an average of 18 minutes and 30 seconds, compared with 8 minutes in the saline control. Lower concentrations (10 mg/mL and 20 mg/mL) still produced clotting times longer than the normal whole blood clotting range of 5–10 minutes, indicating dose-dependent anticoagulant activity of the extract. By contrast, no clot formation occurred in the heparin-treated group, reflecting its potent anticoagulant property .

Table 2: Result of Cohen's D Test on Control and Treatment Groups

	Control	Extract
SD	162.82	177.60
Cohen's D (Effect size)	1.384	

Table 2 reports Cohen's D test comparing the effect size between the fungal extract and the control group. The analysis yielded a value of 1.384, which represents a very large effect size. This confirms that the extract produced a significant difference in clotting time compared to control. Heparin was not included in this calculation, as blood did not clot in its presence and no numerical value could be recorded.

Table 3: Measurements of Thrombolytic Activity of *A. terreus* Extract, Heparin, Water, and Streptokinase

Set up	Trial 1	Trial 2	Trial 3	Average Clot Lysis (%)
10 mg/mL *A. terreus*	30%	32%	26.9%	29.6%
20 mg/mL *A. terreus*	43.5%	38.5%	39.1%	40.4%
30 mg/mL *A. terreus*	46.2%	43.5%	44.8%	44.8%
Heparin	13.8%	9.5%	9.1%	10.8%
Negative Control (Water)	8.7%	8.7%	4.8%	7.4%
Positive Control (Streptokinase)	60%	54.2%	58.3%	57.5%

Table 3 illustrates the thrombolytic activity of the fungal extract at different concentrations, alongside heparin, water (negative control), and streptokinase (positive control). As expected, streptokinase consistently achieved the highest clot lysis, averaging 57.5%. Notably, A. terreus extract at 30 mg/mL also showed strong lytic activity, averaging 44.8% clot lysis, while the 20 mg/mL and 10 mg/mL concentrations produced 40.4% and 29.6% lysis, respectively.

In comparison, heparin demonstrated only 10.8% clot lysis, close to the water control at 7.4%. These findings underscore that, unlike heparin, the fungal extract exerts measurable thrombolytic activity, which increases with concentration .

Table 4: Pairwise Comparison of Thrombolytic Activity using Scheffe Test

	Mean Difference	P- value	Remarks
Extract vs Heparin	30.14	.000	**Difference is significant.** Extract is significantly higher than heparin by 30.14
Extract vs Water	32.63	.000	**Difference is significant.** Extract is significantly higher than water by 32.63.
Extract vs Streptokinase	-14.82	.053	**Difference is nonsignificant.**
Heparin vs Streptokinase	39.80	.000	**Difference is significant.** Streptokinase is significantly higher than heparin by 39.80.
Heparin vs Water	-7.64	.639	**Difference is nonsignificant**
Streptokinase vs Water	47.44	.000	**Difference is significant.** Streptokinase is significantly higher than water by 47.44

Table 4 presents the pairwise Scheffe test comparing thrombolytic activities across groups. The A. terreus extract demonstrated significant differences when compared to both heparin and water, with margins of 30.14% and 32.63% higher clot lysis, respectively.

However, when compared to streptokinase, no significant difference was found, as both treatments showed strong lytic effects, with streptokinase exceeding the fungal extract by only 12.7%. Heparin, on the other hand, showed significantly lower lysis than both the fungal extract and streptokinase. This statistical comparison confirms that the fungal extract's thrombolytic effect is closer to streptokinase than to heparin, highlighting its dual anticoagulant and thrombolytic potential .

Table 5: Evaluation of Prothrombin Time of *A. terreus* Extract and Heparin

Set up	Trial 1	Trial 2	Trial 3	Average Prothrombin Time
10 mg/mL *A. terreus*	34 secs	30 secs	28 secs	31 secs
20 mg/mL *A. terreus*	39 secs	36 secs	29 secs	35 secs
30 mg/mL *A. terreus*	40 secs	36 secs	32 secs	36 secs
Heparin	1 min 27 secs	1 min 21 secs	1 min 30 secs	1 min 43 secs
Control (NSS)	13 secs	12 secs	10 secs	12 secs

Table 5 shows the prothrombin times (PT) of the various groups. The control group exhibited the fastest clotting within the normal PT reference range (10–15 seconds). The fungal extract produced prolonged PTs in a concentration-dependent manner: 31 seconds at 10 mg/mL, 35 seconds at 20 mg/mL, and 36 seconds at 30 mg/mL. Although the differences between 20 mg/mL and 30 mg/mL were modest, both were significantly prolonged relative to control. Heparin again exhibited the strongest effect, with an average PT of 1 minute and 43 seconds, confirming its superior anticoagulant activity.

Table 6: Pairwise Comparison of Prothrombin Time using Scheffe Test

	Mean Difference	P- value	Remarks
Control vs Extract	-20.78	.010	**Difference is significant.** Prothrombin time for extract is significantly higher than control by 11.78 seconds
Control vs Heparin	-64.00	.000	**Difference is significant.** Prothrombin time for heparin is significantly higher than control by 64.00 seconds
Extract vs Heparin	-40.22	.000	**Difference is significant.** Prothrombin time for heparin is significantly higher than extract by 40.22 seconds

Table 6 provides the Scheffe test results for PT comparisons. The fungal extract showed a significant prolongation compared to the control, averaging 11.78 seconds longer clotting time. However, heparin remained significantly stronger, prolonging PT by an additional 40.22 seconds relative to the fungal extract. The results confirm that while A. terreus extract exhibits measurable anticoagulant activity, its efficacy is inferior to that of heparin .

Table 7: Evaluation of Activated Partial Thromboplastin Time of *A. terreus* Extract and Heparin

Set up	Trial 1	Trial 2	Trial 3	Average aPTT
10 mg/mL *A. terreus*	37 secs	39 secs	37 secs	38 secs
20 mg/mL *A. terreus*	42 secs	41secs	42 secs	42 secs
30 mg/mL *A. terreus*	1 min 48 secs	1 min 20 secs	1 min 17 secs	1 min 28 secs
Heparin	2 mins 11 secs	2 mins	1 min 50 secs	2 mins
Control (NSS)	22 secs	23 secs	27 secs	24 secs

Table 7 reports the activated partial thromboplastin time (aPTT) of the treatment groups. The control group alone remained within the normal reference interval of 25–35 seconds. In contrast, all treatment groups exhibited prolonged aPTTs. The

fungal extract demonstrated concentration-dependent effects, with averages of 38 seconds for 10 mg/mL, 42 seconds for 20 mg/mL, and 1 minute 28 seconds for 30 mg/mL.

Heparin produced the most pronounced prolongation, with an average aPTT of 2 minutes, confirming its expected potency. This indicates that the fungal extract exerts anticoagulant effects on the intrinsic and common pathways, although less strongly than heparin .

Table 8: Pairwise Comparison of Activated Partial Thrombplastin Time using Scheffe Test

	Mean Difference	P-value	Remarks
Control vs Extract	-62.89	.005	Difference is significant. Clotting time for extract is significantly higher than the control by 62.89 seconds
Control vs Herapin	-93.67	.001	Difference is significant. Clotting time for herapin is significantly higher than control by 93.67 seconds
Extract vs Herapin	-30.78	.171	Difference is nonsignificant.

Table 8 summarizes the pairwise Scheffe test for aPTT. The fungal extract differed significantly from the control, with clotting times prolonged by 62.89 seconds, supporting its anticoagulant potential. However, heparin was substantially stronger, prolonging clotting by 93.67 seconds compared to control, making it 30.78 seconds more effective than the fungal extract.

These results suggest that while A. terreus extract acts on the intrinsic and common pathways, it remains less effective than heparin, though still capable of significantly extending clotting time relative to untreated samples.

4.2 Discussion

The objective of this study was to assess the anticoagulant and thrombolytic activity of crude Aspergillus terreus extract in vitro, situating its performance alongside unfractionated heparin, streptokinase, and appropriate controls.

Across the assays performed, the results converged on three consistent findings: the extract delayed clot formation in both whole blood and plasma-based cascade assays in a concentration-dependent manner; it demonstrated measurable thrombolytic activity that was significantly greater than negative controls and approximated that of streptokinase; and, as expected, heparin produced the most potent anticoagulant effects while showing no thrombolysis. These findings suggest that A. terreus contains bioactive components with dual anticoagulant and thrombolytic activity, a combination that is not typical of standard clinical agents.

The whole blood clotting time assay provided the most integrative perspective of the extract's activity, as it captures the combined effects of cellular and plasma factors on clot formation. As reported in Table 1, clotting times increased progressively with extract concentration, with 30 mg/mL extending the time to more than double that of the saline control.

This dose-response relationship was not only visually apparent in the data but also statistically supported, with the effect size (Table 2) exceeding conventional thresholds for a large effect. The absence of clotting in the heparin group validated the assay's sensitivity and confirmed the robustness of the control comparisons. These findings establish that even in crude form, the A. terreus extract exerts a measurable anticoagulant effect that scales with concentration.

The thrombolytic assay provided a complementary perspective by examining clot dissolution rather than prevention. Table 3 demonstrated a clear gradient of clot lysis across extract concentrations, from approximately 30% at 10 mg/mL to nearly 45% at 30 mg/mL. By contrast, heparin produced only minimal lysis, close to the water control, while streptokinase achieved the highest lysis at nearly 60%.

Importantly, the Scheffé test (Table 4) confirmed that the extract differed significantly from water and heparin, while the difference with streptokinase was not statistically significant. Although absolute means favored streptokinase, the lack of a significant difference in this relatively small sample set suggests that the extract's thrombolytic effect is of comparable magnitude. This is a key finding: whereas heparin operates exclusively as an anticoagulant, the extract demonstrated dual functionality, slowing coagulation and reducing existing clot mass.

The cascade-specific assays further refined the interpretation by localizing the extract's activity within the coagulation pathways. In the prothrombin time (PT) test, reported in Table 5, the extract prolonged clotting into the 31–36 second range compared with the 12–15 seconds observed in controls. However, the difference between 20 mg/mL and 30 mg/mL was modest, suggesting a plateau effect in the extrinsic and common pathways at higher concentrations.

The Scheffé comparisons in Table 6 reinforced this interpretation: the extract significantly differed from controls but remained markedly less potent than heparin, which extended PT to more than one minute and 40 seconds. This plateau suggests that the active components may have a saturation point

in their ability to disrupt tissue-factor–mediated initiation of clotting.

The activated partial thromboplastin time (aPTT) assay, however, revealed a sharper dose response. As seen in Table 7, while clotting was only modestly prolonged at 10 and 20 mg/mL, the 30 mg/mL extract produced a dramatic increase, extending aPTT to nearly one and a half minutes.

The Scheffé test in Table 8 confirmed significant prolongation compared with controls, though again heparin remained more potent at roughly two minutes. The nonlinear jump between 20 and 30 mg/mL in aPTT, compared with the plateau in PT, suggests that the extract preferentially targets intrinsic pathway factors. Potential targets could include factor IXa, factor XIa, or cofactor complexes that are critical for intrinsic amplification of clotting. This disproportionate effect highlights pathway selectivity rather than uniform inhibition, supporting the hypothesis that multiple active components act within the extract, each with different affinities or targets.

When viewed together, the results from Tables 1 through 8 indicate that A. terreus crude extract possesses a biologically coherent profile: it delays clot initiation and propagation in both intrinsic and extrinsic systems, albeit less strongly than heparin, and it reduces thrombus mass in a manner not observed with heparin but comparable to streptokinase. This dual mechanism makes the extract noteworthy, as it offers both prophylactic and potentially therapeutic antithrombotic effects. Clinically, current therapies are typically bifurcated: anticoagulants like heparin prevent clot extension, while thrombolytics like streptokinase actively dissolve clots. A natural product that exhibits both activities simultaneously may have unique utility, particularly in resource-limited contexts where access to multiple agents is constrained.

Mechanistically, several explanations are plausible. The extract may contain proteolytic enzymes, such as collagenase or serine proteases, that directly degrade fibrin and extracellular matrix components, weakening clot stability. This could account for both the prolonged clotting times and the measurable clot lysis. Secondary metabolites, such as coumarin analogues, are also known from fungal sources and could contribute anticoagulant activity through inhibition of vitamin K–dependent factors. The divergence between PT and aPTT responses suggests that more than one active constituent is involved, with distinct sites of action within the coagulation cascade. The crude nature of the extract, containing a mixture of proteins and metabolites, supports this interpretation.

It is also important to consider the limitations. First, crude extracts do not permit attribution of effects to specific molecules, leaving open the possibility of multiple, even competing, activities. Second, the assays were performed on healthy donor blood, which may not reflect pathological states such as hypercoagulability or thrombosis. Third, while the thrombolysis results were statistically significant compared with controls, the non-significant difference with streptokinase should be interpreted with caution, as the small number of replicates limits statistical power. Finally, no safety assays were performed; crude fungal extracts may include cytotoxic or nonspecific proteolytic components, and these must be characterized before any translational application can be considered.

Despite these limitations, the implications are substantial. The data presented in Tables 1–8 establish a strong rationale for fractionation and purification of A. terreus extracts to isolate active constituents. Such work could reveal novel anticoagulant or thrombolytic agents, or potentially synergistic combinations that achieve dual effects. The dose–response patterns suggest that refinement could yield more potent activity without requiring crude concentrations. Moreover, the demonstration of dual anticoagulant and thrombolytic properties in a single preparation distinguishes this extract from established pharmacologic standards and suggests a novel therapeutic avenue.

In conclusion, the findings confirm that Aspergillus terreus crude extract possesses both anticoagulant and thrombolytic activity in vitro. The anticoagulant effect is evident in whole blood and plasma assays, with concentration-dependent prolongation of PT and aPTT, while the thrombolytic effect is shown in dose-responsive clot lysis approximating streptokinase. While heparin remains the superior anticoagulant and streptokinase the superior thrombolytic, the extract uniquely combines features of both. Future research should focus on fractionation, mechanistic mapping, expanded replication, and safety assessment to determine whether the extract's promising in-vitro profile can be translated into viable antithrombotic therapies.

5. Conclusions and Recommendations

5.1 Conclusions

This study set out to determine the anticoagulant and thrombolytic potential of crude Aspergillus terreus extract in vitro using whole blood clotting, coagulation cascade assays, and clot lysis tests. The findings consistently demonstrated that the extract possesses bioactivity that is both measurable and meaningful. In the whole blood clotting assay, clotting times were prolonged in a clear dose-dependent manner, with the highest extract concentration producing more than a twofold increase relative to controls. The effect size was

confirmed to be large, indicating a robust anticoagulant effect even in crude preparation.

The extract's anticoagulant profile was further defined in plasma-based coagulation assays. Prothrombin time (PT) values were significantly prolonged, though the effect plateaued between 20 mg/mL and 30 mg/mL, suggesting a saturation point in interference with the extrinsic pathway. In contrast, activated partial thromboplastin time (aPTT) results displayed a nonlinear surge at higher concentration, pointing toward a stronger influence on the intrinsic and common pathways. This divergence between PT and aPTT indicates that the extract does not act uniformly across the cascade but may contain components with preferential pathway selectivity. Heparin, as expected, remained the most potent anticoagulant, but the extract nevertheless produced consistent, significant prolongation of clotting time compared to controls.

Equally important, the thrombolytic assay revealed that the extract could break down existing clots, a property not observed with heparin. At 30 mg/mL, the extract achieved nearly 45% clot lysis, significantly higher than water and heparin controls, and not statistically different from streptokinase despite a numerically lower mean. This outcome suggests that the extract harbors enzymatic or proteolytic activity capable of fibrin degradation, potentially mediated by collagenases or other fungal proteases. The dual functionality—both slowing coagulation and promoting thrombolysis—distinguishes the extract from existing clinical agents, which are typically specialized for one role.

Taken together, the results demonstrate that Aspergillus terreus crude extract is biologically active in two dimensions of antithrombotic therapy. It cannot yet rival the potency of purified heparin as an anticoagulant nor match the consistency of streptokinase as a thrombolytic, but it combines features of both in a single preparation. This uniqueness underscores its promise as a natural source of bioactive molecules. At the same time, the crude nature of the preparation and the use of healthy donor samples mean that further work is necessary before translational application can be considered. Nonetheless, the study contributes original evidence to the growing field of fungal biotechnology and provides a foundation for deeper exploration of A. terreus as a potential reservoir of novel anticoagulant and thrombolytic agents.

5.2 Recommendations

Based on these findings, several directions for future research and practical application are recommended.

1. Fractionation and purification of the crude extract. The most urgent next step is to separate and identify the specific bioactive components responsible for anticoagulant and thrombolytic activity. Chromatographic separation, mass spectrometry, and proteomic profiling should be employed to determine whether collagenase, serine proteases, coumarin analogues, or other metabolites underlie the observed effects. This will allow attribution of activity to defined molecules and improve reproducibility.
2. Mechanistic characterization. Beyond identification, functional studies should map the precise mechanisms of action. This includes inhibitor challenge assays (to test which class of protease is active), zymography for enzyme profiling, and interaction studies with coagulation factors or fibrin substrates. Understanding whether activity stems from direct fibrin degradation, cascade inhibition, or platelet interaction is essential for positioning the extract in therapeutic contexts.
3. Replication and statistical strengthening. The thrombolytic results, while promising, were based on a limited number of replicates. Larger sample sizes with expanded trials are needed to confirm whether the extract consistently approaches streptokinase activity. Future work should include power calculations and tighter confidence intervals to validate the robustness of these findings.
4. Safety and toxicity testing. Before any translational consideration, rigorous safety evaluation is necessary. Crude fungal extracts may contain cytotoxic proteins, non-specific proteases, or allergens. Tests for cytotoxicity, hemolysis, immunogenicity, and plasma protein degradation should be prioritized. Only with a favorable safety profile can in-vivo experiments proceed responsibly.
5. In-vivo validation. Once active compounds are identified and safety is confirmed, in-vivo animal models of thrombosis should be employed. These studies will establish whether the anticoagulant and thrombolytic effects observed in vitro translate under physiological conditions. Pharmacokinetics, biodistribution, and effective dosing windows must also be investigated.
6. Comparative benchmarking with clinical standards. Future work should not only compare the extract to heparin and streptokinase but also to newer direct oral anticoagulants and fibrinolytic agents. This broader benchmarking will clarify whether A. terreus–derived compounds can fill gaps in efficacy, safety, or accessibility relative to current drugs.

7. Development for resource-limited settings. If validated, the dual-action profile of A. terreus compounds could hold particular relevance in low- and middle-income contexts where access to multiple pharmacologic agents is limited. A single preparation with both anticoagulant and thrombolytic properties may offer cost and logistical advantages, provided safety and consistency are assured.

Wherefore, this study establishes a compelling case for further exploration of A. terreus in the context of antithrombotic therapy. While crude extract activity is promising, the path forward requires systematic fractionation, mechanistic mapping, rigorous replication, and careful safety evaluation. Only then can its translational potential be realized, either as a novel therapeutic agent or as the basis for next-generation anticoagulant and thrombolytic compounds.

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