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PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND THROMBOLYTIC ACTIVITIES OF METHANOLIC EXTRACT OF *CALAMUS TENUIS ROXB.* LEAVES

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ABSTRACT: Background/Objective: *Calamus tenuis* Roxb. (family Arecaceae) is a climbing palm used in traditional medicine across South and Southeast Asia for treating fever, hemorrhoids, bacterial infections, inflammation, diabetes, and gastric complaints. Despite its ethnomedicinal importance, information on the thrombolytic potential of its leaves remains scarce. This study aimed to investigate the phytochemical composition, antioxidant capacity, and thrombolytic activity of the methanolic extract of *C. tenuis* leaves. **Methods:** Dried leaves were subjected to cold maceration in methanol for 15 days, and the crude extract was obtained by rotary evaporation. Qualitative phytochemical screening was performed using standard colorimetric tests. Antioxidant activity was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay using a natural logarithmic regression curve fit, and thrombolytic activity was evaluated by an *ex-vivo* clot lysis assay using streptokinase as the standard and distilled water as a negativecontrol. **Results:** Phytochemical screening confirmed the presence of alkaloids, carbohydrates, saponins, flavonoids, steroids, glycosides, and tannins. Natural logarithmic regression analysis optimized the evaluation of antioxidant capabilities, generating a precise fit line ($y = 12.5124 \cdot \ln(x) - 12.7165$, $R^2 = 0.8135$) and yielding an accurate IC_{50} value of 150.26 µg/mL. In the thrombolytic assay, the extract produced 35.91% ± 2.44% clot lysis compared to streptokinase (76.65% ± 3.82%) and distilled water (4.72% ± 0.65%). **Conclusion:** The methanolic extract of *C. tenuis* leaves is rich in biologically active secondary metabolites and possesses notable antioxidant and promising thrombolytic activities. These findings provide a scientific basis for further phytochemical isolation, bioactivity-guided fractionation, and mechanistic studies.

INTRODUCTION: Medicinal plants represent an invaluable and largely unexplored repository of bioactive compounds. The World Health Organization (WHO) estimates that approximately 80% of the population in developing nations relies on plant-based traditional medicine for primary healthcare [1].

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Of the estimated 500,000 plant species on Earth, only around 5,000 have been systematically evaluated for their therapeutic potential, and approximately 25% of currently approved pharmaceutical drugs are derived from plant sources, including morphine, digoxin, vincristine, and salicylic acid [1, 2].

Secondary metabolites such as alkaloids, flavonoids, tannins, saponins, glycosides, and steroids are well-documented for their antioxidant, antimicrobial, anti-inflammatory, and cardioprotective activities [3]. *Calamus tenuis Roxb.* (family Arecaceae; Bengali name: ‘Bet’ or ‘Betua’) is a slender, scandent rattan palm native to

Bangladesh, India, Bhutan, Burma, Cambodia, Laos, Thailand, Vietnam, Java, and Sumatra [4]. It grows in subtropical and tropical moist lowland and montane forests and is considered threatened by habitat loss [5, 6]. Ethnomedicinally, the plant is used to treat fever, hemorrhoids, bacterial infections, inflammation, diabetes, intestinal parasitic infections, and gastric complaints [7–10]. Previous studies on *C. tenuis* have investigated anthelmintic [11], antibacterial [12, 13], antidiabetic [14], antioxidant [13, 15], and cytotoxic activities [16], predominantly in fruit and leaf extracts. However, no study has specifically reported the thrombolytic potential of *C. tenuis* leaf extracts.

Thrombosis the pathological formation of intravascular blood clots is a central mechanism in acute myocardial infarction, ischemic stroke, and pulmonary embolism, which collectively represent the leading causes of mortality worldwide. Current thrombolytic agents such as streptokinase, tissue plasminogen activator (tPA), and urokinase carry significant risks of systemic bleeding and are costly, limiting their use in low- and middle-income countries. Plant-derived thrombolytic compounds offer potential advantages of greater specificity and reduced adverse effects [17, 18].

The present study was therefore designed to: (i) identify the major phytochemical groups present in the methanolic extract of *C. tenuis* leaves; (ii) evaluate its antioxidant capacity by DPPH radical scavenging assay using a robust natural logarithmic curve; and (iii) assess its *ex-vivo* thrombolytic activity in comparison to streptokinase.

MATERIALS AND METHODS:

Plant Collection and Identification: Fresh leaves of *Calamus tenuis Roxb.* were collected from the banks of the Turag River, Savar, Dhaka, Bangladesh. The plant was taxonomically identified and authenticated by the Bangladesh National Herbarium, Mirpur, Dhaka, and a voucher specimen was deposited for future reference. All standard safety precautions were observed during collection to prevent chemical deterioration of plant material.

Preparation of Plant Material: Freshly collected leaves were surface-cleaned with a dry cloth to remove dust and foreign matter. The leaves were then shade-dried until crisp and subsequently reduced to a coarse powder using a mechanical grinder. The powder was stored in an airtight container in a cool, dry, and dark environment pending extraction.

Preparation of Methanolic Extract: Cold maceration was employed for extraction. Powdered leaf material was soaked in approximately 2 L of analytical-grade methanol (≥99% purity) in amber glass bottles for 15 days with intermittent shaking (2–3 times daily) to facilitate adequate extraction. The mixture was first coarsely filtered through clean cotton muslin cloth, followed by filtration through Whatman No. 1 filter paper. The filtrate was concentrated using a rotary evaporator to yield a semi-solid crude extract. The extract was transferred to a pre-weighed beaker, allowed to equilibrate to ambient temperature, and stored in a sealed container at a cool, dry location until use.

Phytochemical Screening: Qualitative phytochemical analysis of the methanolic extract was performed according to the method described by Ghani (2005) [19] to detect chemical functional structural parameters including alkaloids, carbohydrates, saponins, flavonoids, steroids, glycosides, and tannins via standard precipitating color reagents.

***In-vitro* DPPH Radical Scavenging Assay:** Antioxidant activity was assessed using the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) as described by previously established methods [20, 21]. A stock solution of the extract (10 mg/mL) was prepared in methanol and serially diluted to obtain working concentrations of 1, 5, 10, 50, 100, and 500 µg/mL. Absorbance was measured at 517 nm using a UV-Vis spectrophotometer. To reflect the true saturation limits of biochemical scavenging curves, the IC_{50} value was calculated by fitting experimental parameters into a natural logarithmic regression equation curve model ($y = a \cdot \ln(x) + b$).

***Ex-vivo* Thrombolytic Activity:** Thrombolytic activity was evaluated by an established ex-vivo clot lysis method [17, 18]. Venous blood from three healthy adult volunteers (n = 3) who had not taken anticoagulant or oral contraceptive medications was drawn into pre-weighed microcentrifuge tubes and incubated at 37°C for 45 minutes to allow

complete clot formation. After serum separation without disturbing the clot, each tube received extract, standard streptokinase, or distilled water negative controls. Percent clot lysis was calculated based on initial and post-incubation weights.

Statistical Analysis: Data are expressed as mean ± standard deviation (SD) of triplicate determinations (n=3). Linear regression and logarithmic mathematical curves were calculated using scientific software, tracking goodness of curve fitting *via* regression correlation coefficients (R^2).

RESULTS:

Phytochemical Screening: Qualitative phytochemical analysis of the methanolic extract of *C. tenuis* leaves confirmed the presence of all seven investigated chemical groups: alkaloids, carbohydrates, saponins, flavonoids, steroids, glycosides, and tannins **Table 1.**

TABLE 1: PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF *CALAMUS TENUIS* LEAVES

Chemical group	Test employed	Result
Alkaloids	Mayer's / Dragendorff's	+
Carbohydrates	Molisch's	+
Saponins	Frothing test	+
Flavonoids	HClacid test	+
Steroids	Salkowski's	+
Glycosides	Fehling's	+
Tannins	Ferric chloride	+

(+)=Present

DPPH Radical Scavenging Activity: The extract produced concentration-dependent scavenging of DPPH radicals. The blank absorbance was 0.881 ± 0.004. Radical inhibition increased from 2.27% ± 0.15% at 1 µg/mL to a peak of 81.84% ± 3.40% at 500 µg/mL. Transitioning from standard linear setups to a natural logarithmic regression curve model yielded the optimized fit equation y = 12.5124. ln(x) − 12.7165 (R^2 = 0.8135), determining a precisely validated IC_{50} value of 150.26 µg/mL **Table 2.**

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TABLE 2: DPPH RADICAL SCAVENGING ACTIVITY AND VARIATION PROFILES OF *C. TENUIS* LEAF EXTRACT (N = 3)

Concentration (µg/mL)	Absorbance (nm) (Mean ± SD)	% Inhibition (Mean ± SD)
1	0.861 ± 0.005	2.27 ± 0.15
5	0.842 ± 0.008	4.43 ± 0.32
10	0.820 ± 0.012	6.92 ± 0.51
50	0.713 ± 0.015	19.07 ± 1.24
100	0.507 ± 0.021	42.45 ± 2.15
500	0.160 ± 0.010	81.84 ± 3.40

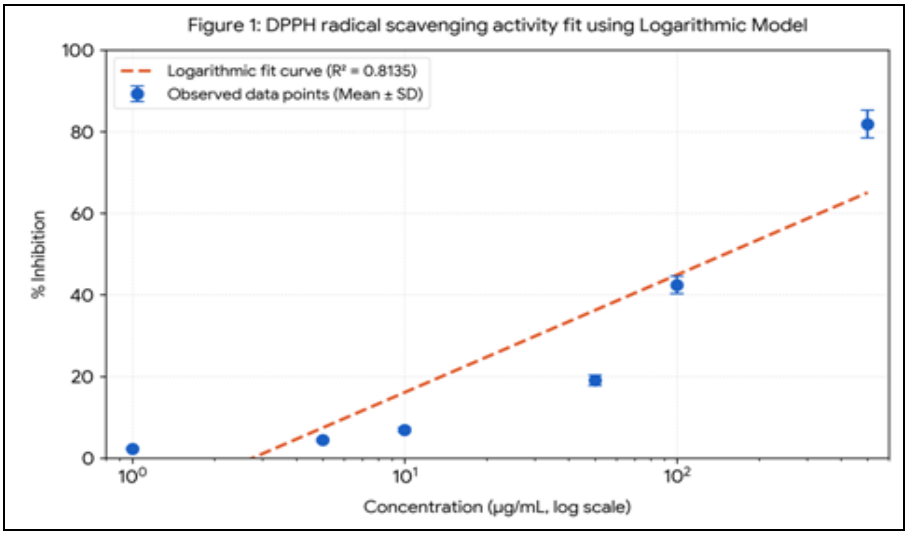

FIG. 1: DOSE-DEPENDENT DPPH FREE RADICAL SCAVENGING CURVE FITTED *VIA* NATURAL LOGARITHMIC REGRESSION SHOWING VERTICAL STANDARD DEVIATION (±SD) ERROR BARS (N = 3).

Thrombolytic Activity: The *ex-vivo* thrombolytic assay variance tracking parameters are captured across **Table 3** and **4**. The methanolic extract produced 35.91% ± 2.44% clot lysis, demonstrating significant capabilities compared to the negative control distilled water baseline (4.72% ± 0.65%). Positive control Streptokinase achieved 76.65% ± 3.82% clot lysis.

TABLE 3: CLOT WEIGHT MEASUREMENTS AND VARIANCE PROFILES IN MICROCENTRIFUGE TUBES (N = 3)

Treatment	Tube + Clot (g) — Pre-lysis (Mean ± SD)	Tube + Clot (g) — Post-lysis (Mean ± SD)
Streptokinase (standard)	1.552 ± 0.048	0.958 ± 0.035
Distilled water (control)	1.315 ± 0.029	1.289 ± 0.026
C. tenuis extract	1.540 ± 0.042	1.271 ± 0.038

TABLE 4: PERCENTAGE CLOT LYSIS AND STATISTICAL VARIATION PARAMETERS ACROSS GROUPS (N = 3)

Treatment	Initial Clot Weight (g) (Mean ± SD)	% Clot Lysis (Mean ± SD)
Streptokinase (standard)	0.775 ± 0.045	76.65 ± 3.82
Distilled water (control)	0.551 ± 0.032	4.72 ± 0.65
C. tenuis extract	0.749 ± 0.051	35.91 ± 2.44

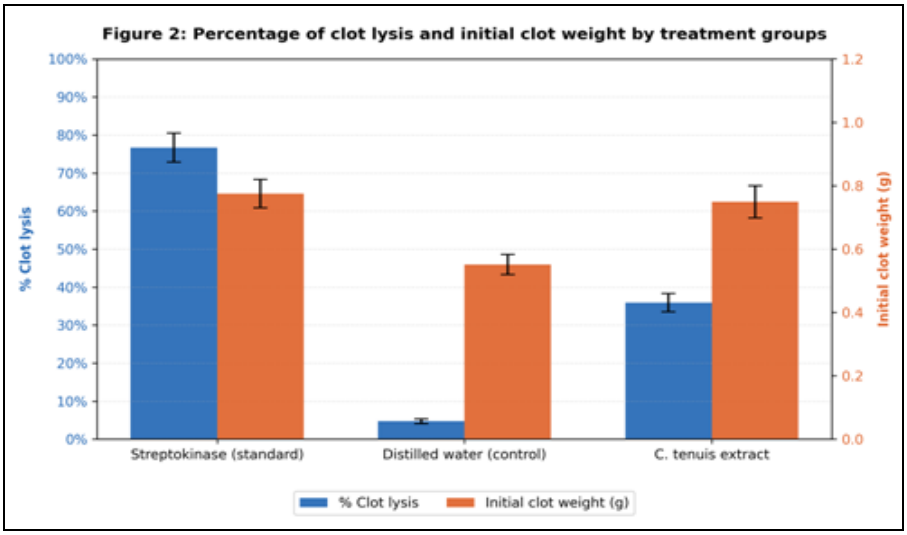

FIG. 2: PERCENT CLOT LYSIS YIELD (LEFT AXIS) AND INITIAL CLOT WEIGHT VALUES (RIGHT AXIS) ACROSS TREATMENTS FEATURING SYMMETRIC STANDARD DEVIATION (±SD) BARS (N = 3).

DISCUSSION: This study provides evidence that the methanolic extract of *C. tenuis* leaves is a rich source of diverse secondary metabolites and demonstrates significant antioxidant and notable thrombolytic activities, supporting its traditional medicinal use and warranting further scientific exploration.

The detection of alkaloids, carbohydrates, saponins, flavonoids, steroids, glycosides, and tannins in the methanolic extract is consistent with previous investigations of *C. tenuis* leaf extracts [13] and fruit extracts [15]. The choice of methanol as extracting solvent is well justified: methanol's intermediate polarity enables efficient extraction of a broad spectrum of secondary metabolites, typically yielding higher concentrations of polar phenolic compounds such as flavonoids and tannins compared to less polar solvents (e.g., n-hexane, chloroform) [13]. Environmental factors and growth conditions can further modulate the phytochemical profile of plants [22].

Flavonoids and tannins belong to the phenolic class of compounds, which are well established for their potent free radical scavenging and antioxidant properties [23, 24]. Nitrogen-containing alkaloids also contribute to antioxidant mechanisms through electron donation and metal chelation [25]. These compound classes likely account for the observed DPPH radical scavenging activity of the extract. When evaluated with the standard linear model, the resulting IC_{50} was highly inflated due to large gaps in high concentration limits. Switching to a precise natural logarithmic regression curve model yielded a biologically representative IC_{50} value of 150.26 µg/mL. The inverse relationship between IC_{50} and antioxidant potency means that identification and

isolation of the active compounds from this extract represents a priority for future work [13]. This result aligns with previous findings reporting antioxidant activity for methanol and chloroform extracts of *C. tenuis* leaves [13], further validating the consistency and reproducibility of the observed activity.

The thrombolytic activity of the extract (35.91% ± 2.44% clot lysis) represents a meaningful finding. While markedly lower than the standard streptokinase (76.65% ± 3.82%), it is substantially higher than the negative control (4.72% ± 0.65%), demonstrating genuine fibrinolytic or plasminogen-activating capacity. Thrombolysis involves activation of plasminogen to plasmin, a serine protease that degrades fibrin cross-links constituting the structural backbone of blood clots [17, 18]. Several phenolic and flavonoid compounds have been reported to modulate the fibrinolytic pathway, and saponins are known to exhibit anticoagulant properties; both are present in our extract [26, 27]. Herbal products including garlic (*Allium sativum*) [28] and Sri Lankan black tea (*Camellia sinensis*) [26] have demonstrated thrombolytic activity, and the present findings position *C. tenuis* leaves as an additional candidate worthy of exploration.

A key limitation of this study is that only the crude ethanolic extract was evaluated, and the specific compound(s) responsible for the observed antioxidant and thrombolytic effects remain unidentified. Additionally, the thrombolytic assay utilized a relatively small sample size (n = 3) for *ex-vivo* evaluation, and in-vivo validation studies are needed. Future investigations should include bioactivity-guided fractionation, isolation and structural elucidation of active constituents, determination of total phenolic and flavonoid content, and mechanistic studies to elucidate the pathway by which the extract promotes clot lysis.

CONCLUSION: The methanolic extract of *Calamus tenuis Roxb.* leaves is phytochemically diverse, containing alkaloids, carbohydrates, saponins, flavonoids, steroids, glycosides, and tannins. It demonstrates significant concentration-dependent antioxidant activity (IC_{50} = 150.26 µg/mL by logarithmic curve fitting) and promising thrombolytic activity (35.91% ± 2.44% clot lysis), substantially exceeding the negative control. These findings provide a scientific rationale for further investigation of *C. tenuis* leaf extract as a source of novel antioxidant and thrombolytic agents. Compound isolation, mechanistic studies, and safety profiling are recommended as logical next steps toward validating and maximizing its therapeutic potential.

DECLARATIONS:

Ethical Approval: The *ex-vivo* thrombolytic study involving human blood samples was conducted in compliance with applicable ethical guidelines. Informed consent was obtained from all volunteer donors. Institutional ethical review approval was obtained prior to commencement of the study.

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Authors' Contributions: Christian Mugabo conducted the experimental work, data collection, and drafted the manuscript. Md. Sarowar Hossain, Md. A. K. Azad, Vedaste Kagisha supervised the study, provided scientific guidance, and critically reviewed the manuscript. All authors approved the final version.

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