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Exploring the Therapeutic Potential of *Musa paradisiaca* L. Pith: A Study on Antioxidant, Cytotoxic, Membrane Stabilizing and Thrombolytic Activities

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Abstract

Musa paradisiaca L. has been utilized for a considerable amount of time in the practice of folk therapies across different cultures, particularly in India. The many components of the plant, including its leaves, roots, and blossoms, are utilized to cure a vast number of conditions, including cough, bronchitis, dysmenorrhea, and menorrhagia. This study investigates the pharmacological potential of different fractions derived from the pith of *M. paradisiaca*, focusing on their antioxidant, cytotoxic, membrane-stabilizing, and anti-thrombolytic activities. The ethyl acetate soluble fraction (EASF) exhibited the highest antioxidant activity, with an IC_{50} value of 18.53 µg/ml, which strongly correlated with its phenolic content. The crude methanol extract (CME) demonstrated remarkable cytotoxicity, with an LC_{50} of 19.91 µg/ml, and significant membrane-stabilizing effects, comparable to aspirin, reducing hemolysis by 74.92% in heat-induced and 44.05% in hypotonic-induced conditions. Moreover, the aqueous and dichloromethane fractions (AQSF and DCMSF) showed promising anti-thrombotic properties, inhibiting clot formation by 28.87% and 20.95%, respectively. These results indicate that, the pith fractions of *M. paradisiaca* possess diverse bioactive properties, particularly CME and EASF, making them promising candidates for developing novel therapeutic agents. Further research is required to isolate the bioactive compounds as well as elucidate their mechanisms of action.

Key words: Antioxidant, cytotoxic, membrane stabilizing, thrombolytic, banana.

Introduction

Conventional medical practitioners from various regions of worldwide rely on medicinal plants to alleviate a wide range of medical conditions (Palombo, 2005). In addition to assisting in the expansion of various medicinal plants to discover the scientific basis for their folk uses, indigenous knowledge passed down through generations in various regions of the globe has played a noteworthy role in developing various traditional medical systems (Jachak and Saklani, 2007). One of the most important factors in the discovery of novel chemical entities has been the growth of naturally occurring substances with biological activity (Hasnat *et al.*, 2023; Newman *et al.*, 2003). The World Health Organization (WHO) states that 80% of the global population depends on herbal medicine as an essential element of primary healthcare (Obonti *et al.*, 2023; Taher *et al.*, 2023). In industrialized nations such as the United States, plant-based pharmaceuticals are projected to include up to 25% of all prescriptions, whereas in rapidly developing countries like India and China, they comprise as much as 80% of pharmaceuticals (Hasnat *et al.*, 2024; Taher *et al.*, 2024). Plants continue to be a significant resource for the development of therapies for critical ailments for instance cancer, oxidative stress, diarrhoea, depression, fever, and thrombosis (Islam *et al.*, 2022). *In vitro* screening approaches have demonstrated efficacy in delivering essential

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early insights required for the selection of crude plant extracts with potential therapeutic qualities for subsequent chemical and pharmacological examination (Shahriar *et al.,* 2024).

The banana plant is a medicinal plant, a member of the Musaceae family, and is cultivated in numerous places worldwide (Shadma *et al.,* 2014). Banana is a tropical fruit and are regarded as the second-biggest fruit product globally (Sharma *et al.,* 2014). The banana, a tropical fruit, has been utilized by humans and animals for generations as a nutritious food source. The flower and stalk of the banana are extensively utilized in culinary practices in southern India (Chan *et al.,* 1998). *Musa paradisiaca* is generally used as a traditional medicine in India for treating many disorders. To cure cough and bronchitis, its leaves can be utilized. The astringent and antihelminthic properties of roots are used to prevent hemoptysis. (Mohammad *et al.,* 2011) Banana fruit has the potential to enhance renal and muscular functions, decrease the potential of renal cancer, and lower blood pressure. It is also utilized to treat diarrhea, stomach aches, lack of appetite, gastric ulcers, mental shock, and to strengthen the immune system and bones. (Swathi *et al.,* 2011). The flower and its stem have antidiabetic properties and are utilized in naturopathy for fat reduction. (Pari *et al.,* 2000; Gomathy *et al.,* 1989). To treat dysmenorrhea and menorrhagia, the flowers' juice and curd are mixed and administered. According to epidemiological studies, the intake of phenolic-rich foods or beverages could mitigate illness risk (Kirtikar *et al.,* 1991).

The banana as peel contains bioactive materials such as flavonoids, tannins, phlobatannins, alkaloids, glycosides, and terpenoids. The pharmacological effect of these bioactive materials is reported, especially as antioxidants, antidiabetics, anti-inflammatory agents, and antibiotics (Chabuck *et al.,* 2013). There is evidence that the juice from banana stems can be used as a natural coagulant to treat wastewater treated with wasted coolant (Habsah *et al.,* 2013). The manganese peroxide enzyme found in banana stem juice is responsible for the enzymatic breakdown of skin melanin. As a result, drinking banana stem juice has several health benefits, including cleansing the body, improving blood flow to the skin, enhancing collagen production, and fighting germs and antioxidants. Furthermore, it adds to a more radiant, younger, smoother, and more attractive appearance (Habsah *et al.,* 2013; Mohorcic *et al.,* 2007). Additionally, banana extracts were found to have wound-healing properties (Agarwal et al. 2009). The banana plant has been used either orally or topically to treat diarrhea, dysentery inflammation, pain, and snakebite therapy, as well as its antilithic properties (Prasad *et al.,* 1993), antiulcerogenic, hypoglycemic (Ojewole *et al.,* 2003), hypolipidemic, antioxidant (Krishnan *et al.,* 2005), and antivenom (Houghton *et al.,* 1992) actions. In the above study, we have seen that different parts of *M. paradisiaca* are utilized to cure various ailments due to their bioactive compounds. The purpose of this research was to determine whether or not the pseudo stem exudate or pith of *Melissoma paradisiaca* L. had any biological effects.

Therefore, this work set out to see whether the methanolic extract and its various solvent fractions of the pith of *M. paradisiaca* could be tested using various *in vitro* models for thrombolytic, antioxidant, cytotoxic, and membrane stabilizing effects.

Materials and Methods

Collection of plant material: The pith of *M. paradisiaca* L. was purchased from a local market and its identity confirmed by a taxonomist (when collected mention please). It was then sun-dried for several days, followed by oven drying at a temperature not exceeding 40°C for 24 hours. Once fully desiccated, the pseudo-stem was ground into a coarse powder using a high-capacity grinder in the Phytochemical Research Laboratory at the State University of Bangladesh.

Plant material extraction: 500 gm of powdered material was placed in a 5-liter cleaned reagent container and steeped in 2.5 liters of methanol for 15 days with occasional shaking and stirring. After 15

days the solution was passed was filtered using Whatman #1 filter paper. Then a rotary evaporator used to concentrate the extract under reduced pressure and temperature. Around 42.8 gm crude methanol extract (CME) of the pith has been yielded (8.56%). After that solvent-solvent partitioning was accomplished by using the Kupchan method, as detailed by Van Wagenen and his team (Van Wagenen *et al.*, 1993). After dissolving 30 gm of pith extract in 10% water, the mixture was extracted using petroleum ether, dichloromethane, and ethyl acetate in that order. Before using any fractions in subsequent experiments, they were all evaporated to remove moisture. After evaporation petroleum ether soluble fraction (PESF; 8.5 gm), dichloromethane soluble fraction (DCMSF, 7.2 gm), ethyl acetate soluble fraction (EASF, 6.4 gm), and aqueous soluble fraction (AQSF; 6.1 gm) yielded.

Evaluation of antioxidant potential

DPPH Scavenging Assay: The DPPH was employed for evaluation of the antioxidant capacity of the methanolic extracts and their various partitionates (Choi *et al.*, 2000). The plant extracts and DPPH samples were mixed in 2.0 ml of methanol solution with concentrations ranging from 500 µg/ml to 0.977 µg/ml. For the next 20 minutes, the solutions were incubated at room temperature in a dark spot. All solutions were measured for absorbance at 517 nm. Using these equations, we were able to determine the percentage of free radical DPPH inhibition (% I).

$$\% I = (1 - A_{sample}/A_{blank}) \times 100\ \%$$

Where,

A_{blank} = Absorbance of the control (containing all reagents except the test material).

A graph was created illustrating the percentage of inhibition compared to the extraction quantity. The IC_{50} value was determined using the formula obtained from the graph.

Determination of Total Phenolic Content: Using the Folin-Ciocalteu reagent as the oxidant and gallic acid as the standard, the total phenolic content of *M. paradisiaca* extractives was analyzed according to the approach described by Skerget *et al.,* 2005 (Majhenic *et al.,* 2007). Distilled water was used to dilute the extractives (2 mg), with a 2 mg/ml concentration. 2.5% w/v Na_2CO_3 and 2.5 ml of Folin-Ciocalteu reagent were added to 0.5 ml of extract solution (2 mg/ml concentration). The mixture was allowed to incubate for 20 minutes at room temperature. The next step was to use a UV spectrophotometer set to 760 nm to test its absorbance. Using gallic acid, a standard curve was created to determine the sample's overall phenolic content.

Anti-inflammatory Activity

Membrane Stabilizing Assay: The membrane-stabilizing efficacy of pith extracts of the *M. paradisiaca* was assessed utilizing the methodology outlined by Sikder et al. (2012), which involved hemolysis induced by heat & cold, as well as hypotonic solution. Blood was collected with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant, washed with isotonic saline (0.9% NaCl), and centrifuged at 3,000 rpm for 10 minutes. The stock erythrocyte suspension was prepared after washing three times. For hypotonic solution-induced hemolysis, the plant extracts (CME, PESF, DMSF, EASF, and AQSF) at 2 mg/ml and the standard aspirin at 0.1 mg/ml were mixed with 0.5 ml of erythrocyte suspension, 4.5 ml hypotonic solution (0.3% NaCl), and sodium phosphate buffer (pH 7.4). The mixture was incubated for 10 minutes at room temperature, centrifuged at 3,000 rpm, and the absorbance was measured at 540 nm. Hemolysis was calculated using the formula:

$$\% \text{ inhibition of hemolysis} = 1 - (OD_2 / OD_1) \times 100$$

In this context, OD_1 represents the optical density of the hypotonic-buffered saline solution alone (control group), while OD_2 denotes the optical density of the test sample in the hypotonic solution.

For heat-induced hemolysis, plant extracts or aspirin were mixed with erythrocytes and isotonic solution, incubated at 54°C for 20 minutes, followed by

centrifugation. Absorbance was measured at 540 nm, and hemolysis was calculated using the formula:

$$\% \text{ inhibition heat-induced hemolysis} = 1 - [(OD_2 - OD_1 / OD_3 - OD_1)] \times 100\ \%$$

Where, OD_1 is the test sample unheated, OD_2 is test sample heated, and OD_3 is the control sample heated.

*Thrombolytic activity***:** *Clot lysis assay:* All of the sample were evaluated for thrombolytic efficacy, with streptokinase (SK) serving as the standard drug (Prasad *et al.*, 2006). 10 mg of extractives were incorporated into 1 ml of distilled water in individual vials. Venous blood (5 ml) from a healthy person was divided into ten pre-weighed sterile Eppendorf tubes (0.5 ml each) and incubated at 37°C for 45 minutes to allow clot formation. The serum was carefully detached, and the clot weight was calculated using the equation:

$$\text{Clot weight} = \text{weight of clot containing tube} - \text{weight of tube alone}$$

Approximately 100 µl of sample solutions were introduced into each Eppendorf tube carrying the pre-weighed clot. In control tubes, 100 µl of SK was added for the positive control, while distilled water was added for the negative control. There was a 90-minute incubation period at 37°C, after which the fluid was drained and the tubes were weighed again to determine the percentage change. The formula was used to determine the percentage of clot lysis:

$$\% \text{ clot lysis} = (\text{Weight of the lysed clot} / \text{Weight of the clot before lysis}) \times 100\ \%$$

Results and Discussion

DPPH scavenging activity: Different extract of Pith of *M. paradisiaca* exhibited different antioxidant value in DPPH scavenging activity assay (Table 1). EASF exert very prominent antioxidant activity with IC_{50} value of 18.53 µg/ml, which was almost reach standard BHT's value of 17.51 µg/ml. Also, PESF and CME manifested notable activity with IC_{50} values of 30.97 and 32.80 µg/ml, respectively.

Table 1. Antioxidant activities of different extractives of pit of *M. paradisiaca* L.

Extract	DPPH scavenging activity (IC_{50}, µg/ml)	Total phenolic content (mg of GAE/g extractive)
CME	32.8	31.26
PESF	139.93	7.10
DCMSF	30.97	24.44
EASF	18.43	75.22
AQSF	101.66	11.93
BHT	17.51	-

Total phenolic content: Table 1 shows that the total phenolic content of the different *M. paradisiaca* pith fractions varied from 7.10 to 75.22 mg of GAE/g of extractives. The plant's phenolic component concentration was highest in the ethyl acetate fraction (EASF), with a considerable proportion also present in the crude methanol extract (CME). Figure 1 illustrates the standard curve of gallic acid employed to ascertain the total phenolic content of several extracts.

Cytotoxic activity: The LC_{50} values of different fractions from the Pith of *M. paradisiaca* were 19.91 µg/ml (CME), 30.13 µg/ml (DCMSF), 42.61 µg/ml (PESF), 59.14 µg/ml (EASF), 68.49 µg/ml (AQSF) and 0.45 µg/ml for the standard (VS), as shown in Figure 2. Among the fractions, CME demonstrated the most potent cytotoxic activity.

Membrane stabilizing activity: Our research showed that CME had impressive membrane-stabilizing effects in hypotonic-induced hemolysis, on par with aspirin's standard effects. Figure 3 shows that compared to the standard Aspirin, CME decreased heat-induced hemolysis by 74.92% and hypotonic solution-induced hemolysis by 44.05%. However, PESFF exhibited significant potential, achieving hemolysis inhibition rates of 68.33% and 30.00% for heat- and hypotonic solution-induced hemolysis, respectively, while EASF showed inhibition rates of 54.60% and 26.06% under the same conditions.

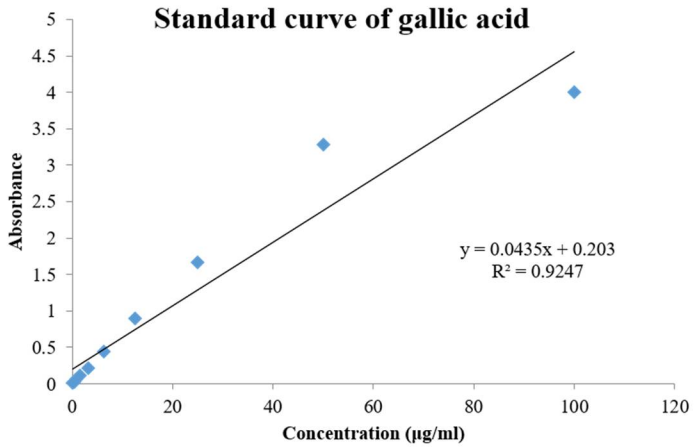

Figure 1. Standard curve of Gallic acid in Total Phenolic Content Assay.

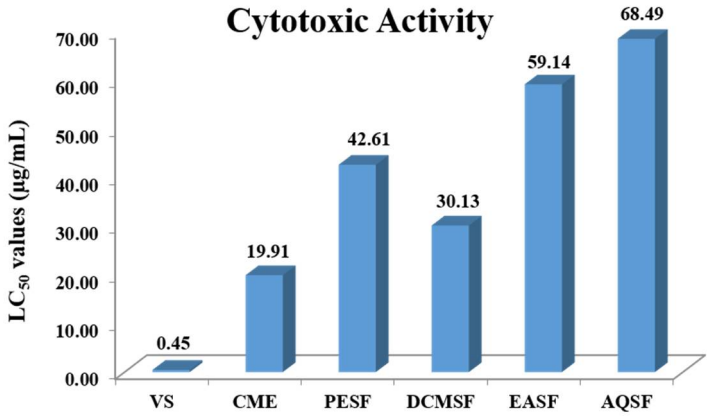

Figure 2. Cytotoxic activities of various fractions of crude methanol extract of pith of *M. paradisiaca*

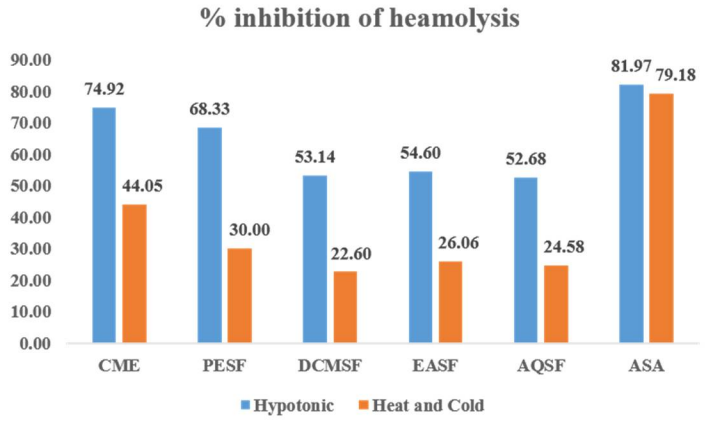

Figure 3**.** Membrane stabilizing activities of various fractions of crude methanol extract of pith of *M. paradisiaca*

Thrombolytic activity: This study demonstrated that the AQSF displayed a potent thrombolytic effect, blocking 28.87% of the clot, comparable to the conventional streptokinase at 30.59% (Figure 4). Furthermore, the DCMSF and PESF fractions exhibited significant anti-thrombotic effects, reducing clot formation by 20.95% and 18.42%, respectively, whereas the control group displayed only 9.35% clot lysis.

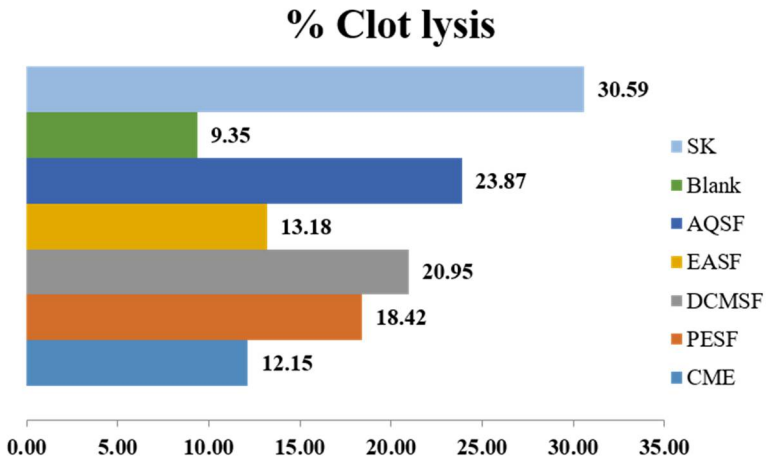

Figure 4. Thrombolytic activities of different fractions of crude methanol extract of pith of *M. paradisiaca.*

Plants serve as a dependable source of essential medicinal compounds (Hasnat *et al.*, 2023; Taher *et al.*, 2024). The search for new bioactive compounds highlights medicinal plants' role in emerging therapies (Obonti *et al.*, 2021; Taher *et al.*, 2023). In developing nations, plant-based remedies are gaining attention for their health benefits, even in underdeveloped areas, relying on traditional medicines (Islam *et al.*, 2022; Zaman *et al.*, 2023). In both modern and traditional medicine, secondary metabolites such as alkaloids, fatty acids, flavonoids, phenolics, and tannins-exhibit ethnopharmacological effects, include analgesic, antidiarrheal, antimicrobial, and antioxidant activities (Alam *et al.*, 2024; Taher *et al.*, 2024b). Research shows that 80% of the 122 plant-derived drug compounds align with their original ethnopharmacological uses (Alam *et al.*, 2024). The aim of the current research was to evaluate the biological and pharmacological potential of the methanolic extract of *M. paradisiaca* pith, confirming its therapeutic significance and offering new insights into its biopotency, thereby supporting its use in traditional medicine.

Polyphenol-rich plant extracts exhibit antioxidant activity by neutralizing free radicals through the scavenging action of their hydroxyl groups (Alam *et al.*, 2021). Flavonoids, in particular, are highly effective at quenching reactive oxygen species and decomposing peroxides and free radicals linked to various diseases (Hasnat *et al.*, 2024). In our study, the ethyl acetate soluble fraction (EASF), dichloromethane soluble fraction (DCMSF), and crude methanol extract (CME) exhibited the highest concentrations of total phenolic content, with values ranging from 14.44 to 75.22 mg of gallic acid equivalents (GAE) per gram of extract (Table 1). This substantial phenolic content is indicative of the potential health benefits of these fractions. The antioxidant capacity of these extracts was further confirmed through the DPPH scavenging assay, which highlighted the EASF as having the highest antioxidant activity, with an impressive IC_{50} value of 18.53 µg/ml. In addition, the PESF and CME also

demonstrated significant antioxidant activity, exhibiting IC_{50} values of 30.97 and 32.80 µg/ml, respectively (Table 1). These findings underscore a robust correlation between phenolic concentration and antioxidant capacity, particularly evident in the EASF fraction. This relationship suggests that the high levels of phenolic compounds in these extracts contribute significantly to their ability to scavenge free radicals, reinforcing the potential of these fractions as sources of natural antioxidants. Such properties are vital for developing therapeutic agents aimed at combating oxidative stress-related disorders.

Cancer is a common condition marked by uncontrolled cell growth and the ability to invade nearby tissues, while cytotoxic compounds can inhibit the spread of cancer cells (Alam *et al.*, 2024). Cytotoxic compounds in plant materials are often evaluated using the brine shrimp lethality assay, which is commonly used for identifying anticancer compounds in human tumors and essential oils. A lower LC_{50} value indicates higher toxicity. Extracts with LC_{50} values above 1,000 µg/ml are considered non-toxic, while those between 500 and 1,000 µg/ml are weakly toxic (Shompa *et al.*, 2024). The evaluation of cytotoxicity across various fractions of *M. paradisiaca* Pith demonstrated a notable range in LC_{50} values, indicating differing levels of potency (Figure 2). The crude methanol extract (CME) emerged as the most effective fraction, showcasing a strong cytotoxic activity with an LC_{50} value of 19.91 µg/ml. This potency suggests that CME possesses compounds that may significantly inhibit cancer cell proliferation. In comparison, the other fractions displayed less cytotoxic activity, with LC_{50} values of 30.13 µg/ml for the dichloromethane soluble fraction (DCMSF), 42.61 µg/ml for the petroleum ether soluble fraction (PESF), 59.14 µg/ml for the ethyl acetate soluble fraction (EASF), and 68.49 µg/ml for the aqueous soluble fraction (AQSF). These values indicate a progressive decrease in cytotoxic potential across the fractions. Notably, the standard (vincristine sulfate, VS) exhibited an exceptionally low LC_{50} value of 0.45 µg/ml, reinforcing its established effectiveness as a cytotoxic agent. The findings from this study highlight the significant potential of CME for further cytotoxic applications, encouraging deeper investigations into its bioactive compounds and their specific mechanisms of action against cancer cells. Understanding these mechanisms could lead to the development of novel therapeutic strategies based on natural products, contributing to the advancement of cancer treatment options.

Inflammation leads to lysosome lysis, releasing enzymes that cause tissue damage. Hemolysis and hemoglobin oxidation occur when RBC membranes rupture due to stressors like a hypotonic environment or heat. Membrane stability prevents protein leakage during inflammation (Shahriar *et al.*, 2024). Our research revealed that the CME exhibited remarkable membrane-stabilizing effects in hypotonic-induced hemolysis, demonstrating efficacy comparable to that of the standard anti-inflammatory agent, aspirin. As illustrated in Figure 3, CME effectively reduced heat-induced hemolysis by 74.92% and hypotonic solution-induced hemolysis by 44.05%, highlighting its potential as an effective anti-inflammatory agent. In addition to CME, the PESF also showed significant membrane-stabilizing activity, with inhibition rates of 68.33% against heat-induced hemolysis and 30.00% against hypotonic solution-induced hemolysis. The EASF similarly displayed noteworthy activity, inhibiting hemolysis by 54.60% and 26.06% in response to heat and hypotonic conditions, respectively. These results suggest that both CME and its derived fractions, particularly PESF and EASF, are promising candidates for further investigation as potential anti-inflammatory treatments. Their ability to stabilize cell membranes under stress conditions points to their therapeutic potential in managing inflammation-related disorders.

A thrombus in blood vessels can obstruct circulation, leading to conditions like hypertension, stroke, and anoxia. Thrombolytic agents help prevent thrombus formation and treat cardiovascular disorders. Research has identified natural compounds from Bangladeshi medicinal plants with anti-thrombolytic potential (Shahriar *et al.*, 2024). This study highlighted the remarkable thrombolytic

potential of the AQSF, which effectively inhibited 28.87% of clot formation, closely matching the efficacy of the standard thrombolytic agent, streptokinase, at 30.59% (Figure 4). Such results underscore the AQSF's potential as a viable alternative for thrombolytic therapies. Furthermore, the study revealed that the DCMSF and PESF fractions also demonstrated significant anti-thrombotic activity, with clot reductions of 20.95% and 18.42%, respectively. These findings are particularly noteworthy given that the control group exhibited only a modest 9.35% clot lysis. Overall, the data suggest that AQSF and its derived fractions could serve as promising candidates for the development of new therapeutic agents aimed at managing thrombotic conditions, warranting further exploration into their underlying mechanisms and bioactive components.

Conclusion

This research manifested the therapeutic potentiality of different fractions derived from the pit of *M. paradisiaca*, demonstrating significant biological activities across various *in vitro* tests. The crude methanol extract (CME) demonstrated the highest membrane-stabilizing and cytotoxic activities, positioning it as a promising candidate for further anti-inflammatory and anticancer research. Additionally, the ethyl acetate soluble fraction (EASF) displayed the maximum antioxidant property, underlining the robust link between phenolic concentration and free radical scavenging ability. Other fractions, including PESF and DCMSF, showed notable cytotoxic, antioxidant, and anti-thrombotic properties, contributing to the overall pharmacological potential of this plant. These findings support the continued exploration of *M. paradisiaca* pit fractions as sources of bioactive compounds, paving the way for the development of novel therapeutic agents. Further research is warranted to isolate and characterize the active constituents responsible for these effects and to explore their mechanisms of action in more detail.

Conflict of Interest

There is no potential conflict of interest between the author.

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