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Cytotoxic and thrombolytic activity of roots of *Musa paradisiaca* (Linn)

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Abstract
The study was aimed to evaluate the cytotoxic and thrombolytic activity of methanol extract of *Musa paradisiaca*. The cytotoxic activity of the crude extract was determined by using brine shrimp lethality bioassay and LC_{50} values of the sample were 22.336 ± 0.41µg/ml whereas for standard vincristine sulfate was 8.50 ± 0.16µg/ml as a positive control. In thrombolytic activity using *in vitro* clot lysis method, the plant's extract showed (46.26 ±1.54%) clot lysis as compared to standard streptokinase (67.32±0.34%).

Keywords: *Musa paradisiaca*, cytotoxic, thrombolytic activity, methanol extract

1. Introduction
Plant-based foods contain significant amounts of bioactive compounds, which provide desirable health benefits beyond basic nutrition. Epidemiological evidence suggests that consumption of a diet rich in vegetables and fruits has positive implications for human health. The World Health Organization reported that 80 % of the world populations rely chiefly on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active constituents [1] and over 25% of modern medicines that are commonly used worldwide contains compounds extracted from medicinal plants [2]. In Bangladesh, there is abundant of medicinal plants and ninety percent of the medicinal plants are wild sourced [3, 4].
During recent decades, there has been an increasing demand for finding newer and safer chemotherapeutic agents. Cancer is the third leading cause of death worldwide, only preceded by cardiovascular disease, infectious and parasitic disease [5]. Extracts of medicinal plants are believed to contain a wide spectrum of polyphenolic, flavonoids, alkaloids, terpenoids and saponin compounds, which might have therapeutic properties and hinder cancer formation [6]. Over 60% of current cytotoxic agents have been derived from natural sources including plants, marine organisms, and microorganisms, either directly or by chemical synthesis based on natural lead compounds [7, 8]. Therefore, natural products have a wide application in cancer chemotherapy [8].
Cardiovascular disease caused by blood clot (thrombus) formation is one among the most severe diseases which are increasing at an alarming rate in the recent years [9]. Homeostasis maintains the integrity of circulatory system after damaging of the vascular channel [10]. Thrombus development is a critical event in the arterial diseases associated with myocardial infarction, anoxia, hypertension [11], stroke, reduction of the blood supply to the liver [12] and venous thromboembolic disorders that account for a considerable number of deaths worldwide [13]. Remarkable efforts have been made towards the discovery and development of natural constituents from various plant and animal sources which have antiplatelet [14, 15] anticoagulant [16, 17], antithrombotic [18] and thrombolytic activity [19-21].
Thrombolytic agents are used to dissolving clot and in the management of thrombosis in patients [22]. Thrombolytic agents such as tissue plasminogen activator (t-PA), Urokinase (UK), streptokinase (SK) [23] etc. are used all over the world for the treatment [24] but their use is associated with hyper risk of haemorrhage [25], anaphylactic reaction and lacks specificity [10, 25]. Because of the shortcomings in the existing thrombolytic agents, a number of researches are underway to improve the variants of these drugs for their better effective nature [26].

Musa paradisiaca (Linn) commonly known as Banana belonging to the family Musaceae [27] is an indigenous plant to Bangladesh. Traditionally the plant was used for different purposes such as abscess, alopecia (female), anasarca, burns, cancer, cataplasm, diabetes, diarrhea, dog bites, dysentery, dyspepsia, corruptions, fractures, gangrene, headache, hematuria, hemiplegia, hemoptysis, hemorrhage, hypertension, lizard bites, mange, marasmus, migraine, nausea, otalgia, psoriasis, ringworm, scorpion sting, septicemia, shingles, smallpox, snake bite, sore, strain, syphilis, tuberculosis, warts, and wound [28, 29]. Pharmacological investigations revealed that banana fruits, Stem juice, flowers are screened for analgesics activity [30], hair growth promoting activity [31], anticonvulsant activity [32], antimicrobial activity [33-38]. The present study was undertaken to investigate the cytotoxic and thrombolytic activity of methanol extract of the roots of this plant.

2. Materials and methods

2.1 Chemicals

Lyophilized streptokinase vial (1500000 I.U.) was purchased from Square Pharmaceuticals Ltd, Bangladesh. Methanol was purchased from Merck, Germany. Normal saline solution was purchased from Beximco Infusion Ltd. All chemicals used were of analytical reagent grade.

2.2 Plant materials

Fresh roots of *Musa paradisiaca* for this study were collected from the local area of Chittagong, Bangladesh and were authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

2.3 Preparation of crude extract

The collected roots were dried for a period of 2 weeks under shade and ground. The ground roots (750 gm) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring. The sediments were filtered and the filtrates were dried at 40 °C in a water bath. The solvent was completely removed by filtering with whatman number-1 filter paper. The solvent was evaporated under reduced pressure at room temperature to yield semisolid. The extract was then preserved in a refrigerator till further use [39].

2.5 Thrombolytic test

This test was performed according to the method described by Prasad *et al.,* 2006 [40]. In the commercially available lyophilized streptokinase vial (1500000 I.U.) 5 mL sterile distilled water was added and mixed properly. This suspension was used as a stock solution from which appropriate dilution was made. Five milliliters of venous blood was drawn from the healthy volunteers (n=10) without the history of oral contraceptive or anticoagulant therapy and was distributed (0.5 mL/tube) to each ten previously weighed sterile microcentrifuge tube and incubated at 37 °C for 45 min to form the clot. After the clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. A volume of 100 µL of methanol extract (10 mg/ mL) was added to each microcentrifuge tube containing pre-weighed clot. As a positive control, 100 µL of streptokinase and as a negative control 100 µL of distilled water were separately added to the control tube numbered. All the tubes were then incubated at 37 °C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. The difference obtained in weight taken before and after clot lysis was expressed as a percentage of clot lysis [41].

2.6 Brine shrimp lethality assay

The assay was carried out according to the principle and protocol previously described by [42-44], with slight modifications. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. Dried cysts of *Artemia salina* were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. After hatching, active nauplii free from egg shells were collected from a brighter portion of the hatching chamber and used for the assay.

The test sample (extract) were prepared by dissolving them in DMSO (not more than 50 µL of 5 mL solutions) plus sea water (3.8% NaCl in water) to attain concentrations of 6.25, 12.5, 25, 50, 100, 300 and 500 µg/ml. A vial containing 50 µL DMSO diluted to 5 mL was used as a control. Vincristine sulfate [45] was used as positive control. After 24 hours the number of survival of nauplii was counted and percentage of mortality was determined using the equation:

% mortality = (no. of dead nauplii/ initial no. of live nauplii) x 100.

The statistical method of probit analysis (Finney's table) [46] was used to calculate LC_{50}. The criterion of toxicity for fractions was established according to (Déciga-Campos *et al.,* 2007) [47]. LC_{50} values > 1000 µg/mL (non-toxic), ≥ 500 ≤ 1000 µg/mL (weak toxicity) and < 500 µg/mL (toxic).

3. Results

The lethality of the crude extract of *Musa paradisiaca* root to brine shrimp was determined on *Artemia salina* after 24 h of exposure the samples, the negative control DMSO and sea water and vincristine sulfate used as a standard. This technique was applied for the determination of a general toxic property of the plant extract. The LC_{50} value (Figure 1) of the extract was 22.336µg/mL and that for standard vincristine sulfate was 8.50µg/mL. No mortality was found in the control group, using DMSO and sea water. The plant extract showed moderate clot lysis activity (46.26%) as compared to standard streptokinase clot lysis (67.32%) activity (Figure 2).

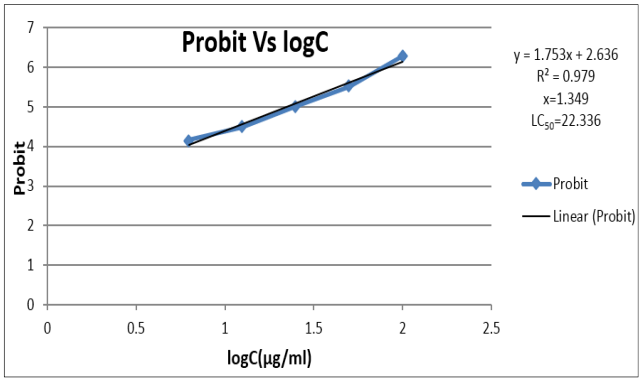

Fig 1: Toxicity assay of *Musa paradisiaca* on brine shrimp. The results are expressed as Mean±SEM of three measurements.

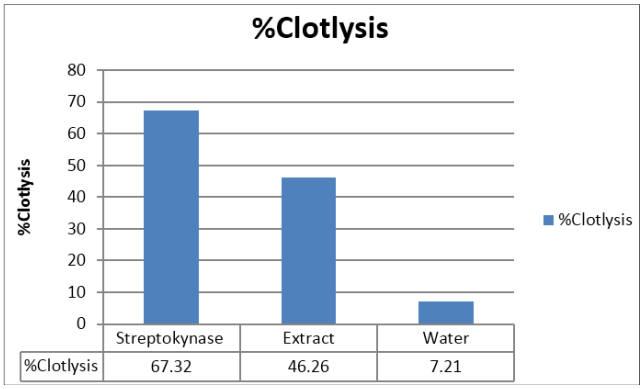

Fig 2: The clot lysis activity of *Musa paradisiaca* extract and streptokinase. All results are expressed as Mean±SEM of three consecutive experiments.

Statistical analysis

All the results obtained by *in vitro* experiment were expressed as Mean±SEM of three measurements followed by Dunnet's test where P<0.01 was considered as statistically significant.

4. Discussion

Most thrombolytic agents work by activating the enzyme plasminogen, which clears the cross-linked fibrin mesh. This makes the clot soluble and subject to further proteolysis by other enzymes and restores blood flow over occluded blood vessels. Thus thrombolytic agents are useful for the treatment of myocardial infarction, thromboembolic strokes, deep vein thrombosis and pulmonary embolism to clear a blocked artery and avoid permanent damage to the perfused tissue (e.g. myocardium, brain, and leg).

Ideally, any agent useful in the treatment of cancer should not be toxic to the normal cell. However, in reality, anticancer agents are often toxic to normal cells, particularly towards rapidly growing cells [48]. It is necessary to test this extract in low concentration to evaluate its potency and also against various cancer cell lines as well as normal cell line so justify the potential to further investigate this plant for anticancer activity.

Hence this study was conducted by crude root extract of *Musa paradisiaca*, further advanced studies should be carried out for compound isolation and it is necessary to observe which compounds are actually responsible for the specific effect.

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