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Research Article

CYTOTOXIC AND THROMBOLYTIC ACTIVITY OF ROOT EXTRACT OF *MUSA PARADISIACA* (FAMILY: MUSACEAE)

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ABSTRACT

Objective: To evaluate for cytotoxicity and thrombolytic activity.

Methods: The cytotoxic activity was investigated by using brine shrimp nauplii. The ethanol extract was prepared by dissolving them in DMSO (not more than 50 µL in 5 mL solution) with sea water (3.8% NaCl in water) using concentrations 6.25, 12.5, 25, 50, 100, 300 and 500 µg/mL. The extract was also evaluated for thrombolytic effect using clot lysis analysis at the concentration of 500µg, 800µg and 1000µg.

Results: The ethanol extract of *M. paradisiaca* showed cytotoxic activity against brine shrimp nauplii and LC_{50} value was 25 µg/ mL and thrombolytic activity was also investigated in our research that the % of clot lysis were 3.27, 7.59 and 13.04 at the concentration of 500µg, 800µg and 1000µg respectively.

Conclusion: This is only a preliminary study. Further studies are necessary to evaluate the exact mechanism behind these activity.

Keywords: *Musa paradisiaca;* ethanol extract; brine shrimp nauplii: cytotoxic Activity; thrombolytic Activity.

INTRODUCTION

The human being appears to be afflicted with more diseases than any other animal species. There can be little doubt then he, very early, sought to alleviate his sufferings from injury and disease by taking advantage of plants growing around him. In the past, almost all the medicines used were from the plants, the plant being man's only chemist for ages. Today, a vast store of knowledge concerning therapeutic properties of different plants has accumulated. All phyla of plants viz. Thallophyta, Bryophyta, Pteridophyta and Spermatophyta contain species that yield official and unofficial products of medicinal importance. (Balick J.M. and P.A. Cox, 1996.)

Many familiar medications of the twentieth century were developed from ancient healing traditions that treated health problems with specific plants. Today, science has isolated the medicinal properties of a large number of botanicals, and their healing components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for use in pharmaceutical preparations."A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." This definition of Medicinal Plant has been formulated by WHO (World Health Organization). The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants which naturally synthesis and accumulate some secondary metabolites, like alkaloids, glycosides, tannins, volatile oils and contain minerals and vitamins, possess medicinal properties.

The fruit of *M. paradisiaca*(Musaceae) is traditionally used in diarrhea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout, hypertension, cardiac disease (Ghani, 2003; Khare,2007).Banana leaves (ashes) are used in eczema (Okoli, 2007), as cool dressings for blister and burns (Ghani, 2003). Flowers are used in dysentery and menorrhagia. Stem juice of fruited plant is used for treating diarrhea, dysentery, cholera, otalgia, haemoptysis and flower is used in dysentery, diabetes and menorrhagia (Ghani, 2003). The root is used as anthelmintic (Khare, 2007), blood disorders, venereal diseases (Ghani, 2003). The plant is also used in inflammation, pain and snakebite (Coe and Anderson, 1999)but according to the best of our knowledge there is not any scientific detailed report on cytotoxic and thrombolytic activities of *M. paradisiaca.* So we have selected the ethanol extract of roots of *M. paradisiaca* to see the cytotoxic and thrombolytic activity.

MATERIALS AND METHODS

Collection and proper identification of the plant sample

The plant was *Musa paradisiaca.* It was collected from Kutubdia at Cox's Bazar district in Bangladesh. The plant was identified by the experts of Bangladesh National Herbarium (BNH), Mirpur, Dhaka and was given an accession number which was 38767. The specimen was preserved in BNH.

Preparation of powdered plant material

The collected root was washed with water, separated from undesirable materials. They were aerated by Fan aeration to be partially dried. Then they were heated through Oven to be fully dried at below 40ºc for two days. The fully dried Leaves were then grinded to make them powder by the help of a suitable grinder. The powder was stored within zipper bag in refrigerator at +4ºc for one month.

Cytotoxic Activity

The brine shrimp lethality bioassay was used to elucidate the cytotoxic compounds using organism *Artemia salina* for the screening. The eggs of the brine shrimp were hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using the method (Meyer *et al,* 1982) which also concurred with the method used (Hossain S *et al,* 2012).The test sample was prepared by dissolving them in DMSO (not more than 50 µL in 5 mL

solution) with 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 negative control. The matured nauplii were applied to each of all experimental vials and control vial. After 24 h, the vials were inspected using a magnifying glass and the number of surviving nauplii in each vial was counted.

Counting of nauplii: After 24-hours of incubation, the vials were observed using a magnifying glass and the numbers of survivors in each vial were counted and the results were noted. From this, the percentage of viability of the nauplii was calculated at each concentration by the following formula:

$$\textbf{\% Nauplii viability} = \frac{N_t}{N_0} \times 100$$

Where N_t = Number of viable nauplii after 24 hrs of incubation, N_0 =Number of total nauplii transferred i.e. 10.The LC_{50} (median lethal concentration) was then determined using Probit analysis.

2.4 Thrombolytic Activity:

The thrombolytic activity test was performed by using 0.5ml of freshly collected blood distributed in each of the different pre weighed and labeled sterile eppendorf tubes and incubated at 37°C for 45 minutes. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight. (Prasad et al, 2006)

Clot weight = Weight of clot containing tube – Weight of tube alone

To each eppendorf tube containing pre-weighed clot, 100µl of ethanol extract of *Musa paradisiaca*, was added as a negative non thrombolytic control, 100µl of distilled water were separately added to the control tubes. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation, supernatant fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight take before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated several times with the blood samples of different volunteers.

% Clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

RESULTS

Brine shrimp lethality bioassay

Table1: Bri**ne shrimp** cytotoxicity of **Ethanol** extract of ***M. paradisiaca***.

Conc. (µg/ml)	LogC	Total	Death	Alive	% of mortality	Probit	LC_{50} (µg/ml)
Blank	-	10	0	10	0	-	
6.25	0.7958	10	2	8	20	4.16	25
12.5	1.09691	10	3	7	30	4.48	
25	1.39794	10	5	5	50	5.00	
50	1.69897	10	7	3	70	5.52	
100	2	10	9	1	90	6.28	
300	2.47712	10	10	0	100	-	
500	2.69897	10	10	0	100	-	

Table 2: Clot lysis Analysis of Negative Control (Water)

Concentrations		Composition of the Tube				
W-1=500µg W-2=800µg W-3=1000µg		100µl Distilled Water + Clot				
Label of the tube	**Empty Weight of The tube(gm)**	**Weight of tube With Clot (gm)**	**Weight of Clot (A gm)**	**Weight of tube with Clot after lysis (gm)**	**Weight of lysis Clot (B gm)**	**% of Lysis (B×100) /A**
W-1	0.92	1.54	0.62	1.53	0.01	1.61
W-2	0.96	1.68	0.72	1.67	0.005	0.69
W-3	0.94	1.69	0.75	1.68	0.01	1.33

Statistical Analysis:

Mean ± Standard Deviation: 1.21 ± 0.4

Table 3: Clot lysis Analysis of *Musa paradisiaca*

Concentrations		Composition of the Tube				
N-1=500µg N-2=800µg N-3=1000µg		100µl Solution of *Musa paradisiaca* + Clot				
Label of the tube	**Empty Weight of the tube(gm)**	**Weight of tube With Clot (gm)**	**Weight of Clot (A gm)**	**Weight of tube with Clot after lysis (gm)**	**Weight of lysis Clot (B gm)**	**% of Lysis (B×100) /A**
N-1	0.94	1.55	0.61	1.53	0.02	3.27
N-2	0.92	1.71	0.79	1.65	0.06	7.59
N-3	0.94	1.86	0.92	1.74	0.12	13.04

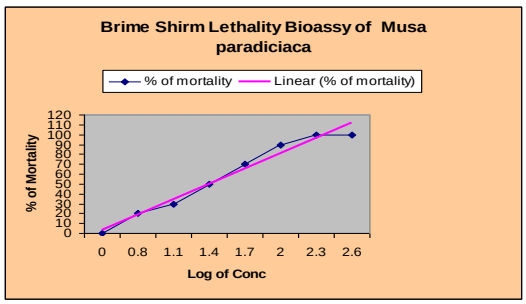

Fig.1: Determination of LC_{50} values for Ethanol extract of root of *Musa paradisiaca* from linear correlation between log concentrations versus % of Mortality.

In-Vitro Thrombolytic Activity Study

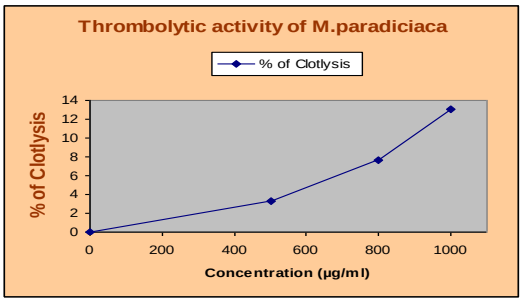

Fig.2: In-Vitro Thrombolytic Activity Study of *Musaparadiciaca*

DISCUSSION

Brine shrimp lethality bioassay is recently developed method in this field. Extracts from plant origin can be tested for their toxicity by this method and the simple zoological organism; brine shrimp nauplii (*Artemia salina,* Leach) are used as a favorable monitor for screening in the discovery of new bioactive natural products. Brine shrimp lethality bioassay (Mclaughlin, 1990; Persoon, 1980) is a rapid and comprehensive bioassay for the bioactive compounds of the natural and synthetic origin. By this method, extracts of natural product as well as the pure compounds can be tested for their bioactivity. The plant extract indicated that different mortality rate at different concentration (Table 1). The mortality rate of brine shrimp napulii was found to be increased with the increase with the concentration of the sample. The median lethal concentration (LC_{50}) was calculated. The LC_{50} value of root of *Musa paradisiaca* was(25µg/ml**).**So, it is evident that ethanol extract of root of *M. paradiciaca* was cytotoxic as well as biologically active. 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 PE to clear a blocked artery and avoid permanent damage to the perfused tissue (*e.g.* myocardium, brain, and leg).Test sample showed different % of Clotlysis at different concentration. The % of Clot lysis was found to be increased with the increase with the concentration of the sample (Table 3 and Figure 2). The ethanol extract of *M. paradisiaca* showed thrombolytic activity.

The experiment shows that the ethanol extract of root of *M.*

paradisiaca has considerable cytotoxicity and thrombolytic activity. Hence this study was conducted by crude extract, further advanced studies should be carried out for compound isolation and it is necessary to observe which compounds are actually responsible for specific effects.

CONCLUSION

In conclusion,the present study, using in vitro experiments established that,In case of anticancer drug preparation this plant extracts may treated as a good candidate as it has notable cytotoxic effect. Ethanol extracts of *M. paradisiaca* possesses cytotoxic activity. In case of thrombolytic activity the extract have appreciable effect. However, Further study is needed to ensure the use of these plant content in human health remedy like Cancer, Cardio vascular disease etc.

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