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Study of cytotoxic and thrombolytic activity of *Phalaris canariensis* in different extracts

Kazi Nuruddin Al Masud[1], Nuzhat Mahbub[2], Tushar Ahmed Shishir[3], Dr. Mahboob Hossain[4], Nasiful Islam[5], Faizul Alam[6], Rezowana Islam[7]

[1, 5, 6, 7] Department of Pharmacy, BRAC University, Dhaka, Bangladesh
[2] Department of Pharmacy, East West University, Dhaka, Bangladesh
[3] Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh
[4] Professor, Department of Mathematics and Natural Sciences, BRAC University, Dhaka, Bangladesh

Abstract
This study was conducted to evaluate the thrombolytic activity and cytotoxic activity of *Phalaris canariensis*. *Phalaris canariensis* has been first time reported in our study. The plant belongs to the Poaceae family and locally used for the treatment of diabetes tumors, boils and buboes. The aqueous, methanolic, ethanolic, ethyl acetate, chloroform and their cyclohexane soluble partitioning materials extracts of *Phalaris canariensis* were used to evaluate the thrombolytic property and the cytotoxic activity. Streptokinase was used as standard for the thrombolytic activity evaluation and the highest % of clot lysis was found in the the aqueous soluble fraction, which was 82.04. Vincristine sulphate was used as standard for cytotoxic activity and the chloroformic extracts provides comparatively best IC50 value which was 94.58 µg/ml.

Keywords: *Phalaris canariensis*, cytotoxic activity, thrombolytic activity, different extracts

Introduction
Medicinal plants are the great source of potential pharmacological properties. Different parts of plant like leaves, roots and barks used to cure many life-threatening diseases. 80% of people used these natural source medicines; around 25% of medicines are came from plants [1]. Approximately, 250,000 plants are angiosperms and gymnosperms available on earth and 500,000 plants are with an upper level [2]. For biological activities 6% plants have been used and for phytochemical analysis 15% plants have been used [3]. These medicinal plants contain with active compounds which provide antiviral, antibacterial, antifungal, anti-inflammatory, anti-helminthic and antioxidant activity [4]. Around 60% of pharmaceutical products manufactured from these medicinal plants [19].

In this study seeds of *Phalaris canariensis* are used and this plant belongs to the family Poaceae. This Poaceae family have different types of medicinal properties such as antioxidant, antimicrobial, cytotoxic. This family also have little DNA damaging activity [8]. The plants from Poaceae used by the tribals of Kinnaur for cold, cough, cuts, wounds, cancer and stomach related problems [9]. Other studies of *Phalaris canariensis* revealed many important pharmacological information. The ether extract from canary seeds showed the highest antioxidant activity [13]. Diterpenes from canary seed have effective antioxidant activity compared to standard antioxidants such as BHT, α-tocopherol, curcumin, BHA and quercetin [5]. Hexane Extract of *Phalaris canariensis* seed have antiobesity effect and it can reduce serum glucose and inhibit insulin resistance [6]. Moreover, canary seed have encoded peptides which showed inhibitory activity against dipeptidyl peptidase (DPPIV) and angiotensin-converting enzyme (ACE); for diabetes and hypertension treatments these enzymes are targets [7]. Furthermore, roasted canary seed flour have higher concentrations of several minerals and vitamins than wheat and it also have more protein. So, it can be a potential source of food [14].

In addition, there is a high chance to found pharmacological properties in the compounds which are present in plants [10]. Compounds which are derive from plant source have significant level of potency and most of the recent anticancer agents are plant derivatives [12]. Plants also can be source of thrombolytic property. Thrombolytic agents able to remove thrombus which cause lacking of blood to cells and tissues [11]. So, plants is the most important part of medicine world. Because of that this study was based on to invastigate the thromobolytic and cytotoxic activity.

Materials and methods
Collection of the seeds
For this research work, the plants *Phalaris canariensis seeds* were collected between the months of July from Chittagong area in 2017. Afterwards, those seeds' sample were identified from National Herbarium of Bangladesh (NHB), Mirpur with a verification number 42432.

Extraction of seed material
The seeds were dried under sun for a few days and finally oven dried to remove all the moisture content. Then the seeds were crushed to coarse consistency. The coarse grains were extracted in a decreasing polarity order. The coarse plant material (900g) was taken and soaked with 1500 ml of

methanol for 3 consecutive days at 25ºC. The extract was filtered and the filtrate was kept for further extraction. In the same manner the filtrate was soaked in different solvents by polarity decreasing order.
Aqueous > Methanol > Ethanol > Ethyl Acetate > Chloroform > Cyclohexane
For every case, the extract was preserved and solvent evaporation was done by using rotary evaporator. Finally, all the extracts of *Phalaris canariensis* were kept under laminar airflow for protecting it from any type of contamination.

Drugs and chemicals
All the chemicals used in this work were collected from prominent vendors and pharmaceutical companies. Methanol was purchased from Active Fine Chemicals Limited, Bangladesh. Dimethyl sulfoxide (DMSO) was purchased from Fisher Scientific, UK. Beacon Pharmaceuticals Limited, Bangladesh provided Vincristine sulphate whereas lyophilized Altepase (Streptokinase) vial was obtained from Sanofi Bangladesh Ltd.

Cytotoxic activity
According to Meyer, Brine shrimp lethality bioassay is a worthy measurement of cytotoxic activity of plant extracts [16]. McLaughlin describes Brine Shrimp lethality test as a cooperative source of other pharmacological assays like antimicrobials, antivirals, pesticides and tumor-resistant, etc. [17]. One liter of water was taken into a small tank and 38g of sea salt were dissolved in it for the purpose of this test and filtered to obtain 3.8% clear solution [18]. *Artemia salina* a specie of brine shrimp was incubated and developed as nauplii in the tank. Test solution was prepared by mixing 4mg of methanolic extract with 100µL of dimethyl sulfoxide in a suitable vial by using vortex mixer. 100µL solution of sample was taken and mixed with 5ml of sea water in a test tube therefore, the concentration obtained was 400µg/ml. Then a series of solution at a concentration of (200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.125 µg/ml, 1.5625 µg/ml, 0.78125µg/ml) were prepared by serial dilution method. Then, 10 live nauplii were added into each test tubes containing 5 ml of sea water along with test sample. After 24 hours, survived nauplii number in each vial was counted by using magniflying glass and the percent (%) of mortality of brine shrimp nauplii was calculated for each concentration.

Thrombolytic activity
Standard drug and test solution preparation
Different concentration of test sample was prepared using crude extract of the seeds of *Phalaris canariensis.* Seed extracts were dissolved methanol and shaken vigorously which were kept overnight and decanted to release supernatant. 2mg/ml, 4mg/ml, 6mg/ml, 8mg/ml and 10mg/ml test sample were prepared respectively which were then filtered through 0.22microne syringe filter to acquire the clear test solution to observe the thrombolytic activity. On the other hand, 30,000 IU Streptokinase (Streptase®, by Sanofi-Aventis) which is a lyophilized Altepase was used as standard drug.

Thrombolytic analysis
A method developed by Daginawala was followed as standard method to observe the thrombolytic activity of this plant [15]. 5 ml of venous blood were drawn from healthy volunteers without a history of oral contraceptive or anticoagulant therapy and drug, which were then dispersed in five different sterile micro centrifuge tubes (0.5 ml/tube) and incubated at 37°C for 45 minutes. As result, blood serum floated and blood cells precipitated at the bottom of the tube. Afterwards, Then the serum was removed from the tubes carefully devoid of disturbing the precipitated cells. Weights of the clots were then calculated (weight of tube with clots – weight of tube alone). Each tube containing the clots were labelled properly and early prepared extracts were introduced into them. Meanwhile, Streptokinase (SK) and distilled water were separately added to the control tubes as positive control and negative control respectively. Then coagulated blood specimen with test samples and control samples were allowed to stand for 90mins at 37ºC. After successful incubation, the fluid generated in each tube were removed and weight variation was calculated (weight of tube and clot – weight after lysis). Thrombolytic activity was presented as the percentage of lysis.

Wt. of tube and clot – wt. of tube tube = wt. of clot
Wt. of tube and clot – weight after lysis = wt. of lysis

$$\frac{wt.of\ lysis}{wt.of\ clot} \text{ x } 100 = \% \text{ of lysis}$$

Statistical analysis
Every analysis was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. For each extract triplicate data was taken and the final data was taken by the triplicate data's mean ± SD (Standard Deviation), which was analyzed by Microsoft excel.

Results and discussion
Cytotoxic activity
Brine shrimp lethality bioassay
Lethal Concentration (LC50) is a standard measure of the toxicity of the substance that kills half of the test nauplii at a specific time which refers to the presence of cytotoxicity activity.

Table 1: LC_{50} values of the test samples of Canary seed and vincristine sulphate

Sample Name	Regression line	R2	LC50 (µg/ml)
Vincristine sulphate	y = 30.799x + 60.653	0.973	0.45
Water	y = 0.086x + 15.546	0.7071	399.03
Methanol	y = 0.151x + 22.448	0.9027	169.78
Ethanol	y = 0.1297x + 2.603	0.7844	278.09

Acetone	y = 0.1309x + 28.699	0.7175	150.75
Chloroform	y = 0.1594x + 32.649	0.7238	94.58
Cyclohexane	y = 0.0533x + 20.86	0.4409	599.04
Pet ether	y = 0.1295x + 20.132	0.6761	222.49

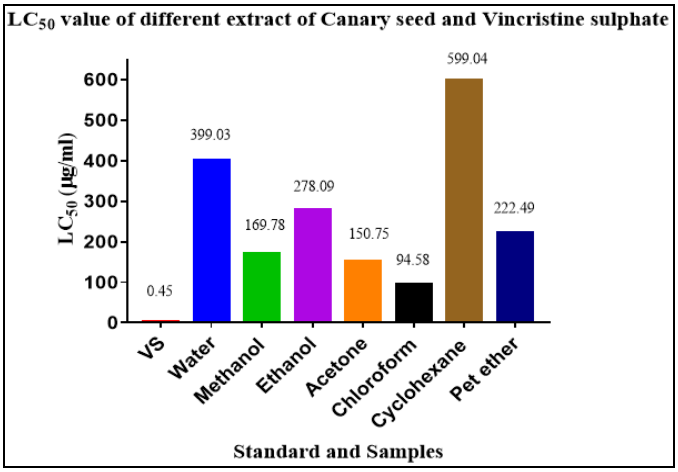

Fig 1: LC_{50} value of different extract of Canary seed and Standard

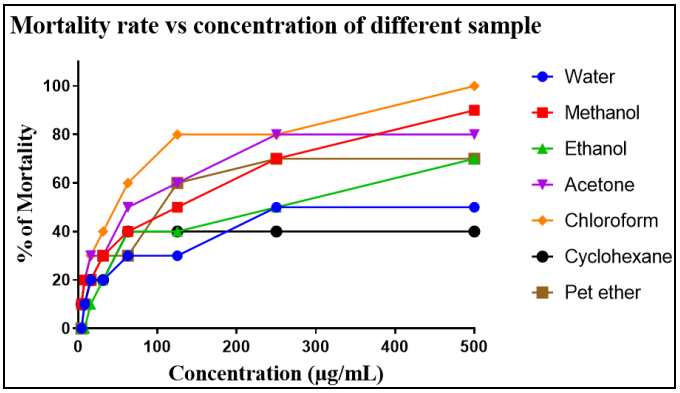

Fig 2: Graphical representation of % of mortality and concentrations of different sample of Canary seed.

In this experiment, as a reference standard vincristine sulphate was used. Here, vincristine sulphate showed the LC_{50} value was 0.45 µg / ml. Different extracts was compared with the standard and among all extractive, chloroform extract showed highest lethality with LC_{50} value of 94.58 µg / ml. Other extractives like water, methanol, ethanol, acetone, cyclohexene, and pet ether were showed LC_{50} respectively 399.03 µg / ml, 169.78 µg / ml, 278.09 µg / ml, 150.75 µg / ml, 599.04 µg / ml, and 222.49 µg / ml (Table 4). This experiment revealed that Canary seed has cytotoxicity activity and different extract showed different level of cytotoxic property. The graphical representation presented the relationship between the percentages of morality and the different concentration samples where the percentage of mortality increases with concentration. Regression line of each sample provided in Table 4. Finally, with proper purification and isolation Canary seed can be used as a cytotoxic agent.

Thrombolytic activity

To identify blood thinning medications from plant source different extractives of *Phalaris canariensis* seeds were studied for thrombolytic activity. All the results were displayed in the table 2.

Table 2: % of clot lysis of different extract of Canary seed and standard.

Sample	% of clot lysis
Blank	4.79
Water	82.04
Methanol	61.12
Ethanol	51.39
Acetone	46.74
Chloroform	32.33
Cyclohexane	37.28
Pet Ether	22.36
Streptokinase	66.77

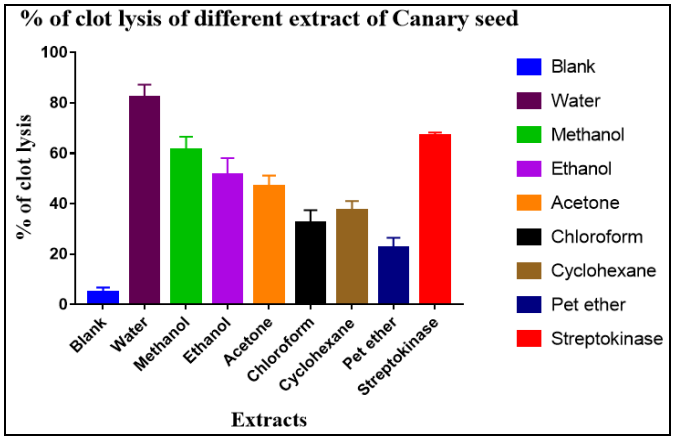

Fig 3: % of clot lysis of different extract of Canary seed and Standard.

In this study, Streptokinase was used as a positive control. The expansion of 100 µl of Streptokinase (30,000 i.u. units) used in coagulation and kept for subsequent incubation for one and a half hours at 37 ° C. After that Streptokinase showed 66.77 % of clot lysis. Here, purified water (Blank) used as negative control which showed very low amount of clot lysis 4.79 %. Clot lysis rate of positive and negative control is much fluctuate from each other. Moreover, water extract showed very astonishing thrombolytic activity which was 82.04 %. On the other hand, pet ether extract showed lowest rate of clot lysis which was 22.36 %. Other extractives such as like methanol, ethanol, acetone, chloroform and cyclohexane showed rate of clot lysis respectively 61.12 %, 51.39 %, 46.74 %, 32.33 % and 37.28 %. This experiment revealed thrombolytic property of Canary seed so with adequate isolation and purification Canary seed can be used as thrombolytic agents.

Conclusion

Brine shrimp lethality bioassay indicated cytotoxic activity of different extract of Canary seed. Among all extractive chloroform showed highest and most potent cytotoxic

property. Furthermore, Canary seed has moderate level of thrombolytic activity and water extract showed highest rate of clot lysis. So, it can be used as both cytotoxic and anti-thrombolytic agent. Crude extract was used in all of the experiments and it is considered as preliminary study.
Finally, more sophisticated investigation is needed to reach a concrete conclusion on the results of this study.

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