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In vitro Clot Lysis Activity of Different Extracts of *Mangifera sylvatica* Roxb. Leaves

Rashaduz Zaman, Mohammad Parvez, Md. Jakaria, Mohammed Abu Sayeed and Minhajul Islam
Department of Pharmacy, International Islamic University Chittagong, Chittagong, 4203, Bangladesh

Corresponding Author: Md. Jakaria, Department of Pharmacy, International Islamic University Chittagong, Chittagong, 4203, Bangladesh

ABSTRACT

The present study was aimed to evaluate the *in vitro* thrombolytic activity of different extracts of *Mangifera sylvatica* Roxb. leaves. An *in vitro* thrombolytic model was used to check the clot lysis effect of seven different extracts (benzene, hexane, petroleum ether, chloroform, methanol, ethanol and ethyl acetate) of *M. sylvatica* along with streptokinase as a positive control and water as a negative control. In the *in vitro* thrombolytic model, benzene, hexane, petroleum ether, chloroform, ethanol and ethyl acetate extracts showed 17.164, 17.384, 22.876, 28.984, 29.206 and 25.226% clot lysis, respectively. Among the extracts methanol extract showed significant percent of the clot lysis (46.934%) with reference to streptokinase (80.514%). From our study it was found that *M. sylvatica* possesses thrombolytic properties that could lyse blood clots *in vitro*; however, *in vivo* clot dissolving properties and active component (s) of *M. sylvatica* for clot lysis are yet to be discovered. Once discovered, *M. sylvatica* could be suggested as a thrombolytic agent in the treatment of patients suffering from atherothrombotic diseases.

Key words: Thrombolytic, streptokinase, clot lysis, different extracts, *Mangifera sylvatica*

INTRODUCTION

Atherothrombosis is the hardening and narrowing (medically known as 'stenosis') of the body's arteries. It is caused by a slow and progressive build-up of plaque under the lining of the arterial wall, which may gradually narrow the artery and restrict blood flow to the target organ. Thrombolysis, also known as thrombolytic therapy, is a treatment to dissolve dangerous clots in blood vessels, improve blood flow and prevent damage to tissues and organs. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue Plasminogen Activator (tPA), the protein that normally activates plasmin. Thrombolytic agents that include tissue Plasminogen Activator (t PA), Urokinase (UK), Streptokinase (SK) etc., are used all over the world for the treatment of atherothrombotic diseases such as myocardial or cerebral infarction, at times leading to death (Mucklow, 1995).

Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and communities in many developing countries (Ghosh, 2003). Herbal products are often perceived as safe because they are "natural" (Demrow *et al*., 1995). Epidemiologic studies proved that foods possessing anti-thrombotic effect could reduce risk of thrombosis. Herbs showing thrombolytic activity have been studied and some significant observations have been reported

(Basta *et al*., 2004). In Bangladesh, in recent years, there has been increasing research on traditional ayurvedic herbal medicines on the basis of their known effectiveness for the treatment of ailments for which they have been traditionally applied (Zaman *et al*., 2015).

Mangifera sylvatica Roxb. is a plant in the 'Anacardiaceae' family. It is found in Bangladesh, Cambodia, China, India, Myanmar, Nepal and Thailand (en.wikipedia.org). The fruit is obliquely ovate, 8-10 cm long, much compressed distally forming a hook and has scantly whitish-yellow pulp which is almost fibreless. Few chemical entities isolated from *M. sylvatica* are friedelin-1, friedelan-3β-ol-23, Cycloartenol-9, Obtusifoldienol-66, β-sitosterol-24 (Anjaneyulu and Radhika, 2000).

As, there is no scientific report available concerning the thrombolytic activity of leaves extract of *M. sylvatica*, thus we aimed to evaluate the thrombolytic activity of various extracts of leaves of *M. sylvatica.* Phytochemical screening was also done to find out the potential chemicals within the plant leaves.

MATERIALS AND METHODS

Plant materials: The leaves of *M. sylvatica* were collected from Chittagong hill tracts area specifically from the area of Department of Forestry, University of Chittagong in May, 2014 and it was authenticated by Dr. Shaikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Preparation of extracts: The leaves of the plant were air dried at room temperature before grinding them to powdered form with the help of mechanical grinder (NOWAKE, Japan). Dried and powdered leaves were soaked separately in different solvents having different polarity (i.e., benzene, hexane, petroleum ether (40-60°C), chloroform, methanol, ethanol and ethyl acetate). Each extract was first filtered through muslin cloth to clarify and then through a Whatman No. 1 filter paper. The filtrate was evaporated under reduced pressure in vacuum evaporator. The dried crude extracts were sterilized overnight by UV radiation and then stored at room temperature in amber color glass vials until used for test of thrombolytic activity.

Reagents and chemicals: All the chemicals and reagents (i.e., benzene, hexane, petroleum ether, chloroform, ethanol and ethyl acetate) were of analytical grade and were provided by the Department of Pharmacy, International Islamic University Chittagong. Streptokinase (1.5 million unit/vial; Sanofi-aventis Bangladesh Limited) were used as positive control and water as negative control for *in vitro* thrombolytic test.

Ethical consideration: The study protocol was approved by the P and D Committee (pharmacy and drug committee-institutional ethics committee), Department of Pharmacy, International Islamic University Chittagong, Bangladesh. Blood samples were collected from the students of the Department of Pharmacy, International Islamic University Chittagong. A written consent was taken from all the volunteers.

Phytochemical evaluation: The leaves powder was dissolved in distilled water and screened for the presence of various chemical constituents like alkaloids, carbohydrates, proteins, cardiac glycosides, steroids, saponins, flavonoids, terpenoid, tannins and phenols (Chowdhury *et al*., 2014; Musa *et al*., 2009; Kokate, 1997; Ugochukwu *et al*., 2013; Yadav and Agarwala, 2011).

Preparation of test solution: Hundred milligram extract was suspended in 10 mL distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant. Hundred microliter of this aqueous preparation of herbs was added to the microcentrifuge tubes containing the clots to check thrombolytic activity.

***In vitro* thrombolytic test:** The thrombolytic activity was evaluated by the method prescribed earlier (Prasad *et al*., 2006). In our study, 100 µL of each fractional extract was used as experimental drug. Five milliliter of blood samples were collected from volunteer and distributed as 0.5 mL of blood into seven separate pre-weighed (W_1) sterile microcentrifuge tubes. The blood samples were collected from ten healthy different volunteers, who did not have contraceptive or anticoagulant therapy for the last 7 days. The blood specimen was incubated for 45 min at 37°C. After clotting of blood, serum was decanted and removed. Then weight of clotted blood (ΔW) was taken by subtracting the pre-weight (W_1) from the weight of clot containing tube (W_2) as, $\Delta W = W_2 - W_1$. The equation for calculating weight of clot is given below:

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

Then 100 µL extracts of each fraction of *M. sylvatica* were added to the clot containing tubes. Similarly, 100 µL of streptokinase was added to clot of standard tube and 100 µL of water was added to the clot of blank tube, those were used as positive and negative control, respectively. Then all the tubes were incubated at 37°C for 90 min and weighed again for getting the weight variation among the pre weight and final weight that was achieved for clot lyses (thrombolysis). The experiment was repeated three times with same blood sample of 10 volunteers. The percentage of clot lysis was calculated using the following equation:

$$\text{Clot lysis } (\%) = \frac{\text{Weight of lysis}}{\text{Weight of clot before lysis}} \times 100$$

Statistical analysis: The significance between percentage of clot lysis by streptokinase and herbal extracts by means of weight difference was tested by one-way ANOVA followed by Dunnett's test analysis. Data is expressed as Mean±SE with 95% confidence interval. The statistical analysis was carried out by GraphPad Prism ver. 5.04.

RESULTS

The phytochemical screening of the plant revealed the presence of alkaloids, carbohydrates, proteins, cardiac glycosides, steroid, phenols, tannins, saponins, flavonoids, terpenoids. Reducing sugar was not present in the extract (Table 1).

Addition of 100 µL SK (streptokinase), a positive control to the clots along with 90 min of incubation at 37°C, showed 80.514% clot lysis. Clots when treated with 100 µL water (negative control) showed only negligible clot lysis (3.098%). The mean difference in clot lysis percentage between positive and negative control was very significant (p value<0.001). After treatment of clots with 100 µL of different extracts of *M. sylvatica*, the highest percentage of clot lysis was in methanol extract with 46.934%, which mean percentage of the clot lysis compared with the negative control was very significant (p-value<0.001). The study also revealed that benzene fraction, ethanol extract, ethyl acetate extract, petroleum ether extract, chloroform extract and

Table 1: Phytochemical screening of leaves extract of *Mangifera sylvatica* Roxb.

Constituents	Results
Alkaloids	+
Carbohydrates	+
Cardiac glycosides	+
Steroids	+
Flavonoids	+
Saponins	+
Protein	+
Terpenoids	+
Phenols	+
Tannins	+
Reducing sugar	-

+: Present, -: Absent

Table 2: Effect of different crude extract on *in vitro* clot lysis along with negative and positive control

Drugs/crude extracts	Clot lysis (%)
Water	3.098±0.554
Streptokinase	80.510±1.483**
Chloroform extract	28.980±5.565**
Ethanol extract	29.210±4.227**
Ethyl acetate extract	25.230±2.489*
Hexane extract	17.380±3.459
Benzene extract	17.160±1.660
Petroleum ether extract	22.880±2.616*
Methanol extract	46.930±6.760**

**Significant at p<0.001, *significant at p>0.05, values compared with negative control, percentage of the clot lysis is represented as Mean± SEM of three independent values

hexane extract showed 17.164, 29.206, 25.226, 22.876, 28.984 and 17.384% of clot lysis, respectively. Statistical representation of the effective clot lysis percentage by different fractions, positive thrombolytic control (streptokinase) and negative control (water) is tabulated in Table 2.

DISCUSSION

From the beginning of civilization, for the treatment of many diseases human are dependable on plants, nowadays phytopharmacological investigation has created a new field of plant derivative drugs discovery, which are effective in remedial of certain diseases and renewed the attention in herbal medicines. It is estimated that about 30% of the pharmaceuticals are prepared from plants derivatives (Leta *et al*., 2002; Gillman *et al*., 1995). Numbers of pharmaceuticals approved by the Food and Drug Administration (FDA) currently have origins to plant sources. A number of plant sources especially several fruits and vegetables have been studied for their supplements having anti-coagulant, anti-platelet and fibrinolytic activity and there is evidence that consuming such food leads to prevention of coronary events and stroke (Emran *et al*., 2015; Ramjan *et al*., 2014). From preliminary phytochemical evaluation it's revealed that this plant extracts contained several chemical constituents and these types of chemical constituents directly responsible for biological effects. In our present *in vitro* preliminary clot lysis test confirmed that, seven different extracts of *M. sylvatica* leaves showed the thrombolytic activity. The maximum clot lysis activity was mostly observed in methanol extract that means methanol soluble compounds are mainly responsible for the thrombolytic activity. In addition, this finding may indicate the possibility of developing novel thrombolytic compounds. Further studies are needed to quantify the amount of chemical constituents present in this plant extract and further studies also help to isolate, characterize the

compounds responsible for thrombolytic activity. In near future it may be implemented as a thrombolytic agent for the improvement of patients suffering from atherothrombotic diseases.

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