Journal of Fundamental and Applied Sciences

ISSN 1112-9867

Available online at http://www.jfas.info

PHYTOCHEMICAL SCREENING AND THROMBOLYTIC ACTIVITY OF CHLOROFORM EXTRACT OF *URENA SINUATA* (L.)

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Received: 2 August 2014 / Accepted: 5 October 2014 / Published online: 31 December 2014

ABSTRACT

The present study was designed to investigate thrombolytic properties of chloroform extract of *Urena sinuata* along with phytochemical study for the presence of phytochemical constituents. The concentrated extracts were collected and allow to air dry for complete evaporation of chloroform. Phytochemical analyses were found to be positive for carbohydrates and gum, reducing sugar, alkaloid, steroid, glycoside and flavonoids. The percent clot lytic activity was compared with water (positive control) and standard enzyme streptokinase (negative control). The mean percent clot lytic activity of chloroform leaf extract of *Urena sinuata* was found 47.89%, which is significant compare with the positive and negative control. The present study suggests that chloroform extract of *Urena sinuata* has significant thrombolytic activity.

Keywords: *Urena sinuate* (L.); phytochemical; streptokinase; clot lytic activity.

1. INTRODUCTION

Different approaches to drug discovery from plants can be enumerated as: random selection followed by chemical screening, random selection followed by one or more biological assays, follow-up of biological activity reports, follow-up of ethno medical (traditional medicine) use of plants, use of appropriate plant parts as such in powdered form or preparation of enriched/standardized extracts (herbal product development), use of a plant product, biologically potent, as a lead for further chemistry, and single new compounds as drugs[1].

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The future of plants as sources of medicinal agents for use in investigation, prevention, and treatment of diseases is very promising[2]. The objective of the later approach is the targeted isolation of new bioactive plant products, i.e. lead substances with novel structures and novel mechanisms of action. *Urena sinuata* L. (Family: *Malvaceae*), locally known as 'Kunjia' in Bangladesh, is a medicinal herb, which has a good reputation in Bangladesh, India and many other countries of the world as a folk medicine for the treatment of a variety of disease such as bronchitis, low back pain, antirheumatic, antipyretic etc. The roots of the plant are considered as emollient, refrigerant and maturant; used as an external application for lumbago (Low back pain**)**. Leaves are prescribed in inflammation of the intestine and the bladder; decoction is given in colic[3]. But till to date, sporadic attempts have been made for the scientific and methodical validation of these traditional claims. The aim of our present work is to investigate the thrombolytic activity of our selected plant extract i.e. chloroform extract of *Urena sinuate* (L.). Thus the plant may be a source of effective herbal drug.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant

The plant *Urena sinuata* was collected from University of Chittagong in July 2011. The plant leaves was collected and identified by taxonomic experts from BCSIR (Bangladesh Center for Scientific and Industrial Research). A voucher specimen that contains the identification characteristics of the plant was submitted to the herbarium for future reference.

2.2. Preparation of plant extract

The fresh *Urena sinuata* plant was washed with water immediately after collection. The collected leaves were chopped into small pieces, air dried at room temperature $(25\pm2)^0$C for about 15 days and ground into powder form and stored in an airtight container. 200 gm powder was macerated in 900 ml pure chloroform for 7 days at room temperature with occasional stirring. 7 days later, chloroform extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure within 50-55^{0}C through rotatory vaccum evaporator (Bibby Sterlin Ltd., England). The concentrated extracts were collected in a Petri dish and allow to air dry for complete evaporation of chloroform. The whole process was repeated three times and finally, 23.649 gm blackish-green

colored, concentrated stem extract was obtained (yield 16.30 % w/w) which was kept in refrigerator at 4^0C[3].

2.3. Phytochemical investigation of *urena sinuata*

The freshly prepared crude chloroform extract was qualitatively tested for the presence of chemical constituents such as carbohydrate and gums, reducing sugars, alkaloids, steroids, glycosides, tannins, flavonoids & saponins. These were identified by characteristic color changes using standard procedures[4-6].

2.4. In vitro thrombolytic activity of *urena sinuata* chloroform extract

A blood clot (thrombus) developed in the circulatory system due to the failure of homeostasis causes vascular blockage and while recovering leads to serious consequences in atherothrombotic diseases such as acute myocardial or cerebral infarction, at times leading to death. Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (t-PA) to dissolve clots. All available thrombolytic agents still have significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and bleeding tendency. Because of the shortcomings of the available thrombolytic drugs, attempts are underway to develop improved recombinant variants of these drugs. Heparin and Aspirin are only moderately efficient for acceleration of lysis and prevention of reocclusion[7], but are safe. More selective thrombin inhibitors and anti-platelet agents are more potent, but their safety remains to be confirmed. Continued investigation in this area will provide new insights and promote progress toward the development of the ideal thrombolytic therapy, characterized by maximized stable coronary arterial thrombolysis with minimal bleeding. Several third generation thrombolytic agents have been developed[8].

3. EXPERIMENTAL PROCEDURE

3.1. Streptokinase

To the commercially available lyophilized streptokinase (SK) vial (Durakinase, Dongkook Pharma. Co. Ltd. South Korea) of 15, 00,000 I.U., 5 ml sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100µl (30,000 I.U.) was used for *in vitro* thrombolysis.

3.2. Specimen

Whole blood (4 ml) was drawn from healthy human volunteers (n = 10) without a history of oral contraceptive or anticoagulant therapy (using a protocol approved by the Institutional Ethics Committee of Central India Institute of Medical Sciences, Nagpur). 500µl (0.5 ml) of blood was transferred to each of the ten previously weighed micro centrifuge tubes to form clots.

3.3. Herbal preparation

100 mg *Urena sinuata* Chloroform 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, which was filtered through a 0.22-micron syringe filter. 100µl of this aqueous preparation of herb was added to the micro centrifuge tubes containing the clots to check thrombolytic activity.

3.4. Clot lysis

4 ml venous blood drawn from healthy volunteers was distributed in three different pre weighed sterile micro centrifuge tube (0.5ml/tube) 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 (clot weight = weight of clot containing tube – weight of tube alone).

To one micro centrifuge tube containing pre-weighed clot, 100µl of aqueous extract of *Urena sinuata* was added. As a positive control, 100µl of SK and as a negative non thrombolytic control, 100µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37^0C for 90 minutes 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. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated 10 times with the blood samples of 10 volunteers.

4. RESULTS AND DISCUSSION

4.1. Phytochemical screening

Phytochemical screening of *Urena sinuata* extract under this study explored the presence of medicinally active secondary metabolites carbohydrate and gum, reducing sugar, alkaloid,

steroid, glycoside and flavonoids. This investigation also indicated the absence of tannins and saponin. These findings with their corresponding results are summarized[9-11], table 1.

Table 1. Observation and Result of Phytochemical screening

Secondary metabolite	Name of the test	Observation	Result
Carbohydrate and Gums	Molish test	Red violet ring was produced	++
Reducing sugar	Fehling's solution test	Brick red color ppt.	++
	Benidict's test	Red color ppt.	++
Alkaloids	Dragendroff's test	Orange brown ppt.	++
	Wagner's test	Reddish brown ppt.	++
	Hager's test	Yellowish ppt.	++
	Mayer's test	Yellow color ppt.	++
Steroids	Salkowski reaction	Red color in chloroform layer	++
	Libermann-Burchard reaction	Light green color	++
Glycosides	Salkowski reaction	Red color in chloroform layer	++
	Libermann-Burchard reaction	Light green color	++
Tannins	Ferric chloride test	No black ppt. was present	--
	Potassium dichromate test	No orange ppt.	--
Flavonoids	Hydrochloric acid test	Red color	++
Saponins	Foam test	No foam production	--

N.B. "++" stands for the presence and "--" indicates the absence of secondary metabolites.

4.2. In vitro thrombolytic activity of *urena sinuata* chloroform extract

Addition of 100 µl Streptokinase (Durakinase, Dongkook Pharma. Co. Ltd, South Korea), a positive control (30,000I.U.) to the clots along with 90 minutes incubation at 37^0C, showed 85.77% clot lysis. On the other hand, clots when treated with 100µl sterile distilled water (negative control) showed only negligible clot lysis which was only 4.70%. The mean difference in clot lysis percentage between positive and negative control was very significant (***p value < 0.001).

But when 100µl *Urena sinuata* chloroform extract was added to 10 different clots, 47.89% clot lysis were obtained and when compared with the negative control(water) the mean clot lysis percentage differences was significant (***p value < 0.001). Percent clot lysis obtained after treating with water, streptokinase and *Urena sinuata* chloroform extract shown in figure.1.

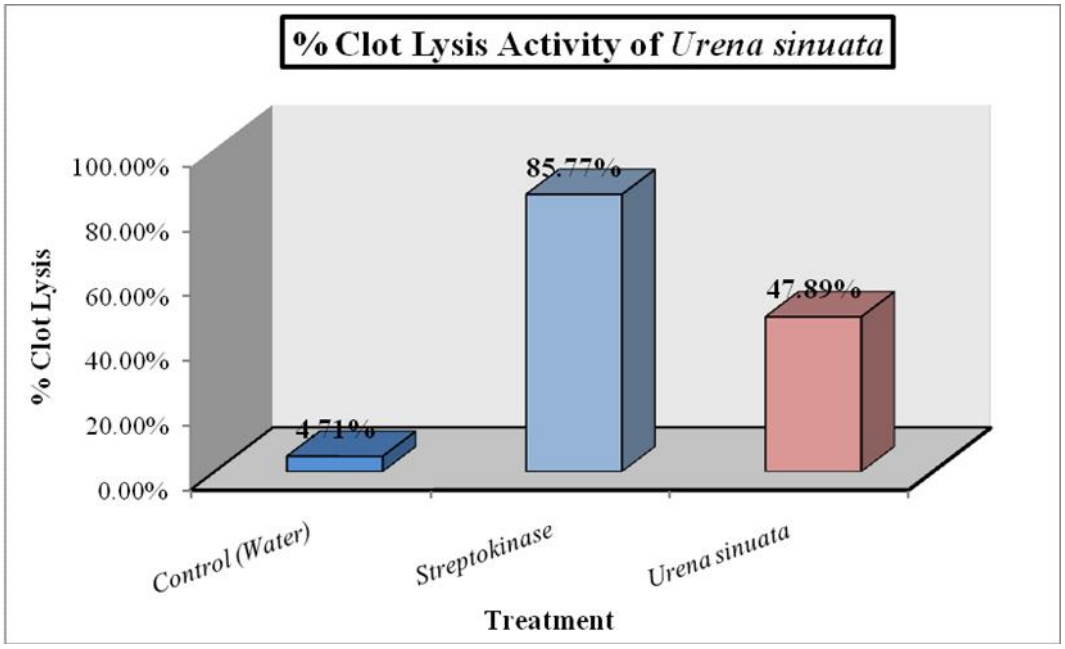

Fig 1. Comparative % *in vitro* thrombolytic effect of *Urena sinuata* chloroform extract, streptokinase and water (negative control)

Statistical representation (*Student's t-test*) of the effective clot lysis percentage by *Urena sinuata* chloroform extract, positive thrombolytic control (Streptokinase) and negative control (sterile distilled water) is tabulated in table 2.

Percentage of clot lysis of 10 different blood samples after treated with water, streptokinase and *Urena sinuata* chloroform extract is shown in table 3.

Table 2. Effect of *Urena sinuata* chloroform extract on *in vitro* thrombolysis

Herbal/ Drug	**% Clot Lysis**
Control (water)	4.70
Streptokinase	85.77
Urena sinuata chloroform extract	47.89

Table 3. Comparing the data of % of Clot lysis using SPSS 11.5 Group Statistics (Control vs. Streptokinase)

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Control	10	4.7990	0.65113	0.20590
	Streptokinase	10	85.7720	1.12211	0.35484

Independent Samples Test

			% of Clot lysis	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		5.950	
	Sig.		0.025	
t-test for Equality of Means	T		-197.372	-197.372
	Df		18	14.444
	Sig. (2-tailed)		0.000	0.000
	Mean Difference		-80.9730	-80.9730
	Std. Error Difference		0.41026	0.41026
	95% Confidence Interval of the Difference	Lower	-81.83491	-81.85038
		Upper	-80.11109	-80.09562

Group Statistics (Control vs. *Urena sinuata*)

Group Statistics

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Control	10	4.7990	0.65113	0.20590
	Urena sinuata	10	47.8840	4.50823	1.42563

Independent Samples Test

			% of Clot lysis	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		10.658	
	Sig.		0.004	
t-test for Equality of Means	T		-29.911	-29.911
	Df		18	9.375
	Sig. (2-tailed)		0.000	0.000
	Mean Difference		-43.0850	-43.0850
	Std. Error Difference		1.44042	1.44042
	95% Confidence Interval of the Difference	Lower	-46.11121	-46.32368
		Upper	-40.05879	-39.84632

Group Statistics (*Urena sinuata* vs. Streptokinase)

Group Statistics

	Control, Streptokinase	N	Mean	Std. Deviation	Std. Error Mean
% of Clot lysis	Streptokinase	10	85.7720	1.12211	0.35484
	Urena sinuata	10	47.8840	4.50823	1.42563

Independent Samples Test

			% of Clot lysis	
			Equal variances assumed	Equal variances not assumed
Levene's Test for Equality of Variances	F		7.667	
	Sig.		0.013	
t-test for Equality of Means	T		25.789	25.789
	D_f		18	10.111
	Sig. (2-tailed)		0.000	0.000
	Mean Difference		37.8880	37.8880
	Std. Error Difference		1.46913	1.46913
	95% Confidence Interval of the Difference	Lower	34.80148	34.61944
		Upper	40.97452	41.15656

Here, all values are expressed as MEAN (n=10).

***p<0.001 significant compared to negative control.

Here, Clot weight = weight of clot containing tube – weight of tube alone.

All the tubes were incubated at 37^0C for 90 minutes and observe for clot lysis. After Incubation fluids released was removed and tubes were again weighted to observe the difference in weight after clot disruption. Differences obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was carried out with blood sample of 10 volunteer.

5. CONCLUSION

The chloroform extract of *Urena sinuata* has significant thrombolytic activity. The mechanism of action and active compounds of *Urena sinuata* is unknown. In the present study, studied on chemical properties was carried out on *Urena sinuata* (Family: *Malvaceae*). Traditional records proved that Malvaceae plants produce diverse classes of pharmacologically active compounds

and some of the Malvaceae species used as emollient, refrigerant and maturant; used as an external application for lumbago. Leaves are prescribed in inflammation of the intestine and the bladder; decoction is given in colic. Infusion of the flowers is used in bronchitis. This is only a preliminary study. Chemical group tests were analyzed qualitatively on *Urena sinuata* chemical group test showed that carbohydrate and gum, alkaloid, glycosides, flavonoids and reducing sugar were present in the chloroform extract of *Urena sinuata.*

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How to cite this article:
Dibyajyoti P. Swati S. Phytochemical screening and thrombolytic activity of chloroform extract of *urena sinuata (l.)*. J Fundam Appl Sci. 2014, *6(2), 210-219.*