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Original Article

Screening of *in-vitro* Thrombolytic and Membrane stabilizing activities of methanolic extract of *Launaea sarmentosa*

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ABSTRACT

The present study was undertaken to analyze in vitro thrombolytic and membrane stabilizing activities of methanolic extract of *Launaea sarmentosa. L. sarmentosa* is a plant of Asteraceae family. Crude methanolic extracts of *L. sarmentosa* at numerous conc. were used for in vitro thrombolytic and membrane stabilizing studies. Just in case of thrombolytic study, it absolutely was dose dependently enhanced, where 10 mg/ml concentration most importantly showed 22.57% lysis of clot ($p< 0.001$) by in vitro clot lysis assay methodology. Streptokinase was used as standard & distilled water was treated as negative management. Crude methanolic extracts of *L. sarmentosa* dose dependently enhanced in membrane stabilizing activity, whereas 10 mg/ml concentration most significantly showed 12.11% & 20.23% inhibition of haemolysis severally by each hypotonic solution and heat induced haemolysis. Acetyl salicylic acid was used as standard in membrane stabilizing study.

Key words: *L. sarmentosa,* Asteraceae, thrombolytic, membrane stabilizing, streptokinase, acetyl salicylic acid.

INTRODUCTION

Medicinal plants offer reasonable means that of health look after poor and marginalized people. Medicative plants are moving from brink to thought use with a larger range of individuals seeking remedies and health approaches free from adverse effects caused by artificial chemicals [1]. Medicinal plants are employed by the traditional physicians for treatment and disease-treating formulations from ancient time [2]. In developed countries thromboembolic disorders such as pneumonic emboli, deep vein occlusion, strokes and heart attacks etc. are the main causes of morbidity and mortality [3]. Thrombolytic therapy uses medication referred to as thrombolytic agents, like alteplase, anistreplase, streptokinase, urokinase, and tissue plasminogen activator (TPA) to dissolve clots. Inflammation is one of the vital pathological disorder. It is a neighborhood of non-specific immune response that happens in reaction to any sort of bodily injury, is a complex biological response of vascular tissues to harmful stimuli [4,5]. Since earlier period, Medicinal plants are used for the treatment of many diseases. The leaves, stems, barks, flowers and underground elements of medicinal plants are most frequently used for ancient medicines. *L. sarmentosa* is a coastal plant. The plant is obtainable in Asian country, South Africa, coastal Madagascar, Seychelles, Mauritius, India, Srilanka, Indo-China and Java. The hunt of the present study was to research the methanolic extract of *L. sarmentosa* for thrombolytic and membrane stabilizing activities.

MATERIALS AND METHODS

Collection of plant materials and solvent extraction: The whole plants of *L. sarmentosa* were collected from Darianagar, CoxsBazar, Bangladesh. The native name of *L. sarmentosa* is Vortashak. Its accession number is 38312, that was confirmed by the National Herbarium Institute, Mirpur, Dhaka, Bangladesh. Once assortment of whole plants of *L. sarmentosa* were completely

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washed with water. Then the collected plant materials were chopped, dried, and powdered. Regarding 500g of the fine materials was soaked in 1.5 litre of methanol at room temperature for two weeks. Then the solution was filtered using filter cloth and Whatman's filter paper and targeted with a rotary evaporator. It rendered a brown granular. The brown granular was selected as crude methanolic extract.

Thrombolytic activity:

Standard drug- Streptokinase (SK): Commercially obtainable lyophilized Altepase (Streptokinase) vial (Beacon pharmaceutical Ltd) of 15, 00,000 I.U., was collected and 5 ml sterile distilled water was added and mixed properly. This suspension was used as a standard from that 100µl (30,000 I.U) was used for *in vitro* thrombolysis [6].

Preparation of test sample: Five totally different test solutions were accustomed to measure the thrombolytic activity of the plant extract. The plant extract was dissolved in methanol and agitated vigorously on a vortex mixer to prepare different concentrations (2, 4, 6, 8 and 10 mg/ml respectively) of the test sample. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22 micron syringe filter. 100 µl of themethanolic extracts were added to the micro-centrifuge tube containing the clots to examine thrombolytic activity [6].

Thrombolytic potential: Thrombolytic activity of *L. sarmentosa* was carried out by in vitro clot lysis assay technique [6]. Aliquots (5 ml) of venous blood were drawn from healthy volunteers without a history of oral contraceptive or anticoagulant therapy, which were distributed in five completely different pre weighed sterile micro centrifuge tubes (0.5 ml/tube) and incubated at 37 °C for 45 minutes. When clot formation, the serum was fully removed without distressing the clot and every tube having clot was again weighed to work out the clot weight (clot weight = weight of clot containing tube – weight of tube alone). Each micro-centrifuge tube containing clot was properly labeled and 100 µl of the test samples from varied concentration (2, 4, 6, 8 and 10 mg/ml respectively) were adscititious to the tubes consequently. As a positive control, 100 µl of streptokinase (SK) and as a negative control, 100 µl of distilled water were individually added to the control tubes. All the tubes were then incubated at 37 °C for 90 minutes and ascertained for clot lysis. When incubation, the fluid was removed and tubes were again weighed to observe the distinction in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% of clot lysis = (wt. of released clot / wt. of clot before treatment) × 100

Membrane stabilizing activity

Preparation of test sample: Different concentration of the methanolic extracts of *L. sarmentosa*as (2, 4, 6, 8, & 10 mg/ml respectively) were prepared as the test samples for membrane stabilizing activity.

Drug: Standard Acetyl Salicylic Acid (ASA) or Aspirin was used as standard drug for comparison with methanolic extracts of whole plants of *L. sarmentosa*.

Red Blood Cells (RBC) Collection: 5 ml of whole blood was collected from healthy human volunteers in a test tube containing an anticoagulant (EDTA 2.2 mg/ml of blood) under standard conditions of temperature 23±2°C and relative humidity 55±10%.

Preparation of Phosphate Buffer Solution: A buffer is an aqueous solution that has a highly stable pH. A pH of about 7.4 with buffer strength of 10 mM was obtained using 0.0352% monosodium phosphate dehydrate and 0.1099% disodium phosphate anhydrate. The buffer was made by adding 0.352 gm monosodium phosphate dehydrate and 1.099 gm disodium phosphate anhydrate to 1000 mL water.

Preparation of Isotonic Solution: A solution that has a concentration of electrolytes, nonelectrolytes or a combination of the two that will exert equivalent osmotic pressure as that solution with which it is being compared. Either 0.16M sodium chloride (NaCl) solution (approximately 0.95% salt in water) or 0.3M nonelectrolyte solution is approximately isotonic with human red blood cells. For the preparation of 500 ml isotonic solution of 154 mM strength, 4.5045 gm NaCl was added and mixed.

Preparation of Hypotonic Solution: A solution of lower osmotic pressure than that of a reference solution or of an isotonic solution is called hypotonic solution. For the preparation of 500 ml hypotonic solution, having strength of 50 mM, 1.4625 gm NaCl was added and mixed.

Erythrocyte Suspension: For the preparation of erythrocyte suspension, the collected RBC was centrifuged, supernatant was removed and the blood cells were washed three times with isotonic solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4) through centrifuge action for 10 min at 3000 rpm using the same volume as

supernatant . Finally it was resuspended in the same volume of this isotonic buffer solution.

Effect on Haemolysis: The following two methods were used to investigate the in vitro membrane stabilizing assay [7,8].

Hypotonic Solution- Induced Haemolysis: 0.5 ml of this erythrocyte suspension was mixed with 5 ml hypotonic solution (50 mM NaCl) containing either the different concentration of methanolic extract (2, 4, 6, 8 & 10 mg/ml respectively) or Acetyl Salicylic Acid (0.10 mg/ml).The Acetyl Salicylic Acid was used as a reference standard. 0.5 ml of RBCs mixed with hypotonic buffered saline alone to consist the control sample. The mixture was incubated for 10 minute at room temperature, then centrifuged for 10 min at 3000 rpm and finally the optical density of supernatant was measured at 540 nm. The percentage inhibition of haemolysis was calculated by the following equation;

% inhibition of haemolysis = $\{(OD_{control} - OD_{test\ sample})/OD_{control} \} \times 100$

Heat induced haemolysis: Aliquots (5 ml) of the isotonic buffer, containing different concentration of the methanolic extract were put into two duplicate sets of centrifuge tubes [7]. The vehicle, in the same amount, was added to another tube as control. Erythrocyte suspension (30 µL) was added to each tube and mixed gently by inversion. One pair of the tubes was incubated at 54 ˚C for 20 min in a water bath. The other pair was maintained at 0-5 ˚C in an ice bath. The reaction mixture was centrifuged for 3 min at 1300 g and the absorbance of the supernatant was measured at 540 nm using UV spectrometer. The percentage inhibition or acceleration of hemolysis in tests and was calculated using the following equation:

% inhibition of haemolysis = $100 \times \{(OD_2- OD_1)/ (OD_3-OD_1)\}$

Where,

OD_1 =test sample unheated,

OD_2 = test sample heated and,

OD_3 = control sample heated.

Statistical analysis: The results are expressed as mean ±SD. Statistical comparisons were made using Dunnett's test. Significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Thrombolytic activity: The effects of *L. sarmentosa* on in-vitro clot lysis are tabulated in Table-1. From table-1, it is pronounced that the percentage of clot lysis was 45.49% when 100 µl of streptokinase (30,000 I.U.) was used as a positive control, while in case of water (negative control) the percentage of clot lysis was negligible (6.06%). The percentage of clot lysis was dose dependently increased, whereas10mg/ml concentration most significantly showed 22.57% lysis of clot ($p< 0.001$).

Table 1: Effects of different concentration of methanolic extracts of *L. sarmentosa* and the control on in vitro clot lysis.

Treatment	W1	W2	W3	% of clot lysis (mean± S.D)	P- Value
ME (2mg/ml)	0.8268	0.1305	0.1249	4.29±0.046	< 0.16
ME (4mg/ml)	0.8318	0.1498	0.1344	10.28±0.074	< 0.08
ME (6mg/ml)	0.8259	0.1640	0.1396	14.87±0.086	< 0.07
ME (8mg/ml)	0.8134	0.1579	0.1293	18.11±0.006	< 0.01
ME(10mg/ml)	0.8267	0.1639	0.1269	22.57±0.009	< 0.01
Blank (water) and Streptokinase					
Treatment	W1	W2	W3	% of lysis	P- Value
SK	0.8275	0.3833	0.2089	45.49±0.068	< 0.001
Blank	0.8167	0.3263	0.3066	6.03±0.077	

Level of Significance ***= $P < 0.001$, **= $P < 0.01$, *= $P < 0.05$

W1 = Weight of vial alone; W2 = Weight of clot containing vial; W3 = Weight of clot containing vial after clot disruption; SK =Streptokinase

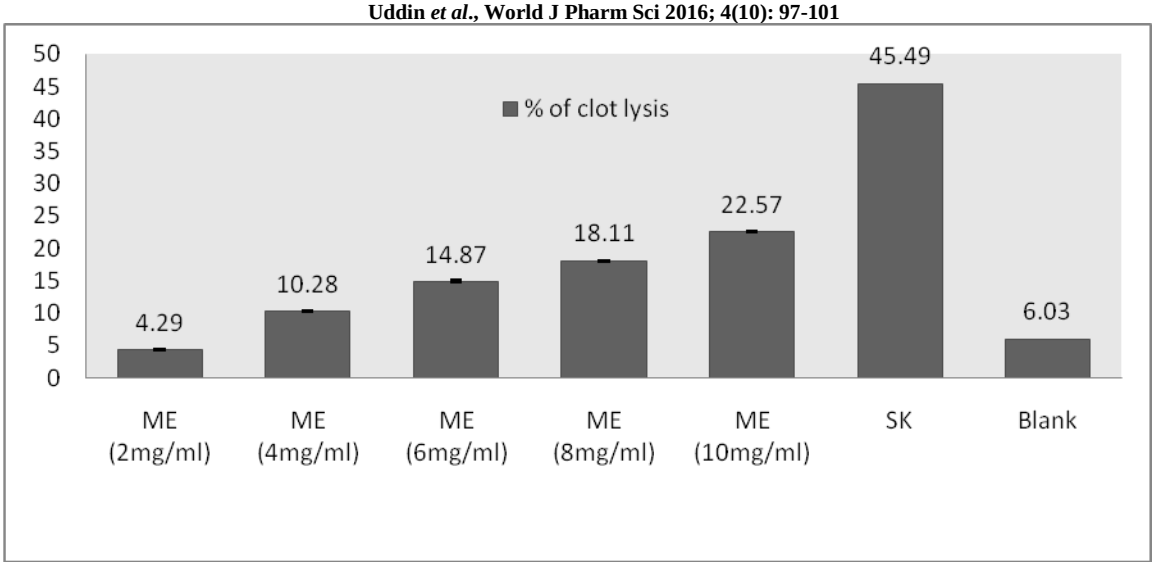

Figure 1: Thrombolytic activity of methanolic extracts of *L. sarmentosa* at various concentration. Here, ME= methanolic extract, SK= streptokinase.

Membrane stabilizing activity: The membrane stabilizing activities of the Crude methanolic extracts of *L. sarmentosa* are showed in Table 2& 3. The crude methanolic extracts dose dependently increased in membrane stabilizing study, whereas 10 mg/ml concentration most significantly showed 12.11% & 20.55% inhibition of haemolysis respectively by hypotonic solution and heat induced haemolysis. Acetyl salicylic acid was used as standard in membrane stabilization.ASA (0.10 mg/mL) revealed 70.01%& 56.32% inhibition of haemolysis respectively induced by hypotonic solution and heat induced haemolysis correspondingly.

CONCLUSION

This study corroborated that the methanolic extracts of *L. sarmentosa* at higher concentration have moderate in-vitro thrombolytic & membrane stabilizing activities.

Table 2: Effect of different conc. of methanolic extract of L. *Sarmentosa* on hypotonic solution-induced haemolysis of erythrocyte membrane.

Treatment	Concentration	Optical density of samples in hypotonic solution (Mean ± SD)	% inhibition of haemolysis
Control	_ _ _	3.698±0.0053	
ME	2 mg/ml	3.396±0.0037	8.16±0.049
ME	4 mg/ml	3.357±0.004	9.22±0.036
ME	6 mg/ml	3.325±0.0019	10.08±0.044
ME	8 mg/ml	3.287±0.0027*	11.11±0.029
ME	10 mg/ml	3.250±0.019**	12.11±0.037
Acetyl salicylic acid	0.10 mg/ml	1.109±0.0045***	70.01±0.017

Table 3: Effects of different conc. of methanolic extract of *L. sarmentosa* on heat induced haemolysis of erythrocyte membrane.

Treatment	Concentration	OD of sample ±SD		% inhibition of Haemolysis
		Heated Solution	Unheated Solution	
Control	- - -	1.093±0.035		
ME	2 mg/ml	0.879±0.107	0.856±0.011	9.7±.069%
ME	4 mg/ml	0.780±0.004	0.743±0.017	10.57±.043%
ME	6 mg/ml	0.715±0.014	0.650±0.022	14.67±.062%
ME	8 mg/ml	0.587±0.082	0.461±0.342*	19.93±.036%
ME	10 mg/ml	0.405±0.008	0.227±0.021**	20.55±.087%
Acetyl Salicylic Acid	0.10mg/ml	0.672±0.025	0.129±0.029***	56.32±0.228%

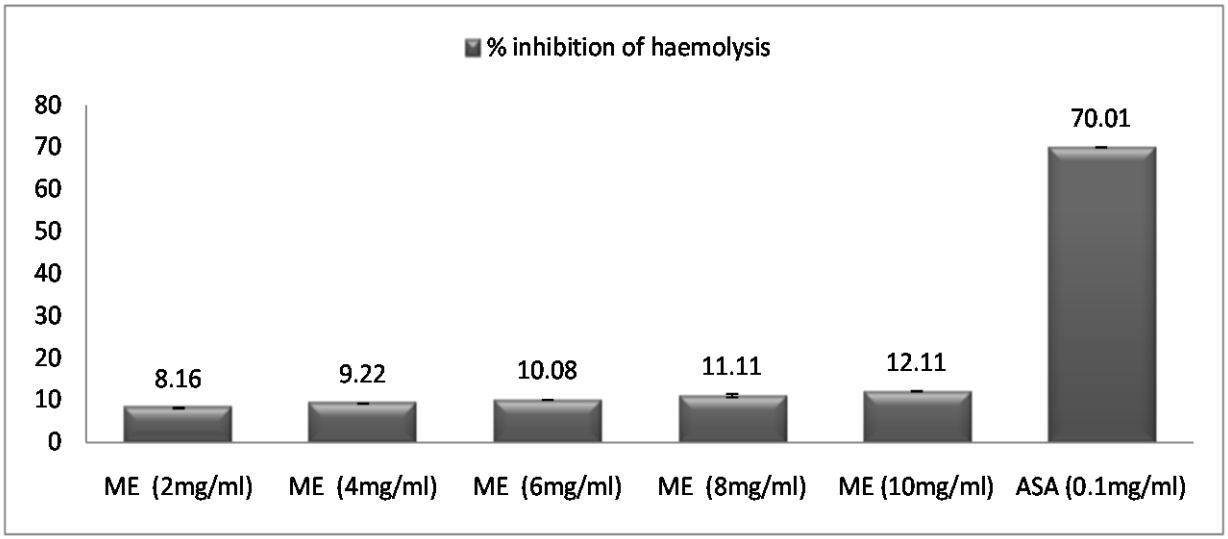

Figure 2: Effect of different conc. of *L. sarmentosa* on hypotonic solution induced haemolysis of erythrocyte membrane.

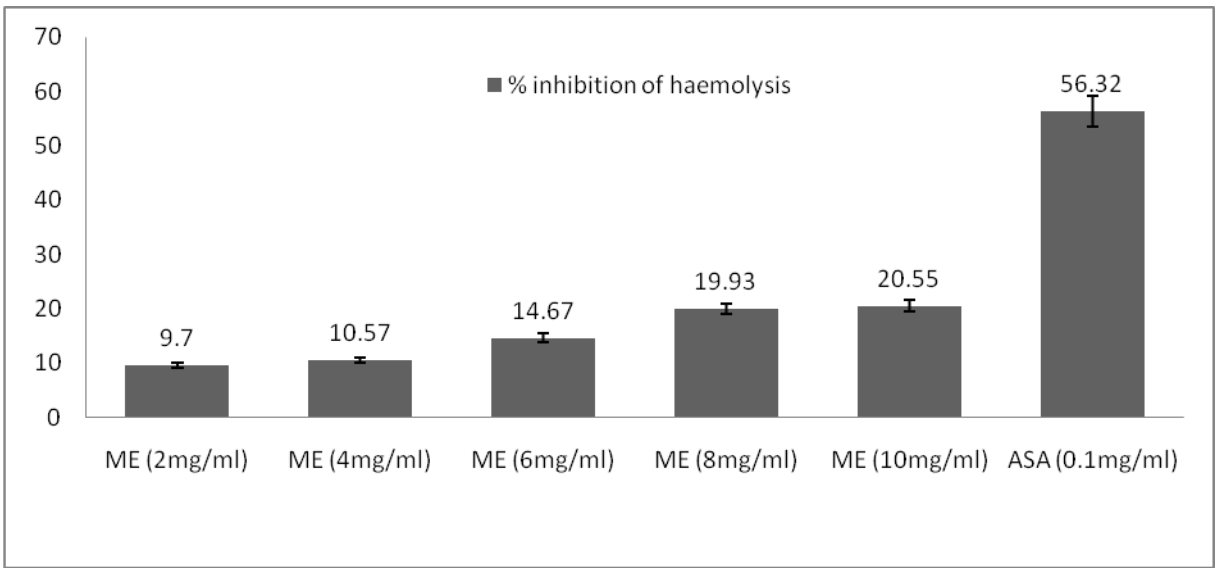

Figure 3: Effect of different conc. of *L. sarmentosa* on heat induced haemolysis of erythrocyte membrane.

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