

**IN-VITRO INVESTIGATION OF THROMBOLYTIC AND
MEMBRANE STABILIZING ACTIVITY OF CISSUS ADNATA IN
DIFFERENT FRACTIONS**

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ABSTRACT

This study was conducted to evaluate the thrombolytic activity and membrane stabilizing activity of *Cissus adnata* (Roxb.). *Cissus adnata* (Roxb.) has been first time reported in our study. The plant belongs to the vitaceae family and locally used for the treatment of tumors, boils and buboes. The methanolic, petroleum ether, dichloromethane, chloroform and their aqueous soluble partitioning materials extracts of *Cissus adnata* (Roxb.) were used to evaluate the thrombolytic property and the membrane stabilizing 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

44.32. Ascorbic acid was used as standard for membrane stabilizing activity and the petroleum ether fraction provides comparatively best % inhibition of hemolysis (heat induced) which appears to be 351.

KEYWORDS: Thrombolytic activity, Membrane Stabilizing activity, Methanol, *Cissus adnata* (Roxb.).

Article Received on
08 Feb. 2018,

Revised on 29 March 2018,
Accepted on 19 April 2018,

DOI: 10.20959/wjpr20189-11948

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INTRODUCTION

Since ancient times, herbal preparations have been used for the treatment of several diseases. Herbal products are often thought to be safe because they originated from natural sources.^[1] Agents from plant sources supposed to be none or less antigenic are also available at cheaper rate. Considerable efforts have been noticed in the discovery and development of natural drugs from both herb and animal sources that have anti-platelet,^[1] anticoagulant^[0] and thrombolytic activity.^[3] World Health Organization (WHO, 2011) stated that nearly (65-80) % people around the world directly relying on medicinal plants for primary treatment. The development of new products from natural sources is also encouraged because it is estimated that of the 300,000 plant species that exist in the world, only 15% have been evaluated to determine their pharmacological potential.^[4] *Cissus adnata* (Roxb.) has been first time reported in our study for evaluation of several pharmacological activities. The other species of *Cissus* genus has been proven to possess certain activities. *Cissus araloides* provides antimicrobial property.^[0] *Cissus hypoglauca* can be used for the treatment of sore throat.^[6] *Cissus rotundifolia*^[0] and *Cissus sicyoides*^[8] both provides anti-diabetic property. Considering the several pharmacological activities provided the different species of *Cissus* genus, the plant *Cissus adnata* (Roxb.) has been chosen for the investigation of Thrombolytic activity and Membrane Stabilizing activity.

Since antiquated circumstances, home grown arrangements have been utilized for the treatment of a few illnesses. The leaves as well as twigs, stem, bark and underground parts of plants are regularly utilized for conventional medications. Home grown items are frequently seen as sheltered in light of the fact that they seem to be "common".^[9] Cerebral venous sinus thrombosis (CVST) is a typical issue which joined by huge bleakness and mortality.^[10] Heparin, an anticoagulating operator, is the primary line of treatment for CVST, as a result of its viability, wellbeing and attainability.^[11] Thrombolytic drugs like tissue plasminogen activator (t-Dad), urokinase, and streptokinase and so on assume an essential part in the administration of patients with CVST.^[0] In this way, the point of the present review was to research the thrombolytic movement of methanolic concentrate and its distinctive portion of entire plant of *Cissus adnata*.

MATERIALS AND METHODS

Plant collection and identification

The plant was collected from Bikrampur in Munshiganj district beside Dhaka city in February, 2017. After the collection of the plant, it was identified by Bangladesh National Herbarium providing the Accession ID 45961.

Chemicals

Dichloromethane, Petroleum ether, Chloroform, Methanol, NaCl, Streptokinase, Acetyl Salicylic Acid were used.

Preparation of plant extract

After proper washing the whole plants were sun dried for a couple days. The dried plant were then ground to a coarse powder and extracted by soaking it in 2.5 liter of methanol. The mixture was kept for 15 days and occasional stirring was maintained. The whole mix was then filtered through a perfect cotton plug ultimately with a Whatman No.1 filter paper. The volume of the filtrate was then diminished using a Rotational evaporator at low temperature and weight. The heaviness of the dried extract was 40gm.

Preparation of sample

The thrombolytic action of all extractives was assessed by a strategy utilizing streptokinase (SK) as standard substance. The dry unrefined concentrate (100 mg) was suspended in 10 ml of refined water and it was kept overnight. At that point the solvent supernatant was tapped and separated.

Streptokinase (SK)

Commercially available Streptokinase vial (Beacon pharmaceutical Ltd) of 15, 00,000 I.U., was collected and 5 ml sterile refined water was included and blended appropriately. This suspension was utilized as a stock from which 100 μ l (30,000 I.U) was utilized for in vitro thrombolysis.

Blood Sample

Entire blood (n=10) was drawn from solid human volunteers without a background marked by oral prophylactic or anticoagulant treatment and 1ml of blood was transferred to the previously weighed tubes and kept for the formation of clot.

Thrombolytic activity

Aliquots (5 ml) of venous blood were drawn from sound volunteers which were circulated in five distinctive pre-weighed clean smaller scale rotator tube (1 ml/tube) and brooded at 37°C for 45 minutes. After clump development, the serum was totally evacuated without aggravating the coagulation and each tube having cluster was again weighed to decide the coagulation weight (clump weight = weight of cluster containing tube – weight of tube alone).

To each smaller scale axis tube containing pre-measured clump, 100µl watery preparations of various fractions alongside the rough concentrate was included independently. As a positive control, 100µl of streptokinase (SK) and as a negative non-thrombolytic control, 100µl of distilled water were independently added to the experimental tubes. Every one of the tubes were then incubated at 37°C for one hour thirty minutes and looked for clot lysis. After incubating, the discharged of liquid was evacuated and tubes were again weighed to determine the change in weight after clot lysis. After determining the difference, then percentage of clot lysis was measured. Process of that calculation given below:

$$\% \text{ of clot lysis} = (\text{wt. of released clot} / \text{clot wt.}) \times 100$$

Membrane stabilizing activity investigation

Studies have revealed that several herbal derived drugs have been demonstrated to contain principles that possess ability to facilitate the stability of biological membranes when exposed to induced lyses. Membrane stabilizing effects encompasses the inhibition or total elimination of action potentials from being propagated across the membrane.^[Error! Reference source not found.]

During the oxidative stress, various reactive oxygen species (ROS) like superoxide, hydroxyl and peroxy radicals are generated. These ROS play important role for the pathogenesis of several health problems like cancer, Alzheimer's disease, cellular aging, diabetes and inflammation.^[15]

The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane.^[15] Therefore, as membrane stabilizes that interfere in the release and or action of mediators like histamine, serotonin, prostaglandins, leukotrienes etc.^[17] Along these lines, the point of the present review was to research the calming action of methanolic concentrate and its distinctive division of entire plant of *Cissus Adnata*.

Preparation of the sample

Table 1: Preparation of different samples

Plant part	Sample code	Test Sample	Concentration
Whole plant of <i>Cissus adnata</i>	Hypotonic medium	-----	50 mM
	ME	Methanolic extract	1 mg/mL
	PESF	Petroleum ether soluble fraction	1 mg/mL
	DCMSF	Dichloromethane soluble fraction	1 mg/mL
	CSF	Chloroform soluble fraction	1 mg/mL
	AQSF	Aqueous soluble fraction	1 mg/mL
	ASA	Acetyl salicylic acid	0.10 mg/mL

Solvent used: Analytical grade

Drug

Standard Acetyl Salicylic acid (ASA) or Ibuprofen was utilized as standard medication for examination with various methanolic concentrates of entire plant of *Cissus adnata*.

Red Blood Cells (RBC) collection

Human RBCs were collected for the review. RBCs taken from individuals who were in 70 kg weight and free from infections. The collected RBCs were kept in a test tube with anticoagulant EDTA under standard condition (temperature $23\pm 2^{\circ}\text{C}$ and relative humidity $55\pm 10\%$).

Effect on Haemolysis

Erythrocyte suspension

Whole blood was collected from rats under standard condition. Heparin was used to prevent clotting. The blood was washed three times with 0.9% saline. The volume of saline was measured and reconstituted as a 40% (v/v) suspension with isotonic buffer solution (pH 7.4).

Heat induced Haemolysis

Portions (5ml) of the isotonic buffer containing 1.0mg/mL of various extractives of plant was put into two copy sets of axis tubes.^[17] The vehicle was in the same amount and was added to another tube as control. Erythrocyte suspension (30 μ L) was added to each tube and mixed gently by inversion. One pair of the tubes was hatched at 54°C for 20min in a water bath. The other pair was kept at $(0-5)^{\circ}\text{C}$ in an ice bath. The reaction mixture was centrifuged for 3 min at 1300g and the absorbance of the supernatant was measured at 540nm.

The rate hindrance or, speeding up of haemolysis in tests was figured by the accompanying condition

$$\% \text{ Inhibition of haemolysis} = 100 \times [1 - (\text{OD}_2 - \text{OD}_1 / \text{OD}_3 - \text{OD}_1)]$$

Where, OD_1 = test sample unheated, OD_2 = test sample heated & OD_3 = control sample heated.

RESULTS AND DISCUSSION

Thrombolytic activity

To discover cardio defensive medications from regular sources the extractives of *Cissus adnata* were evaluated for thrombolytic movement and the outcomes are displayed in table 1.2. Expansion of 100 μ l SK, a positive control (30,000 i.u.), to the coagulations and ensuing brooding for an hour and a half at 37°C, indicated 66.77% lysis of cluster. Then again, refined water was dealt with as negative control which showed an unimportant rate of lysis of clump (36.09%). The mean distinction in cluster lysis rate amongst positive and negative control was discovered exceptionally critical. In this experiment, aqueous soluble fraction demonstrated most astounding thrombolytic action (42.32%). In any case, alternate partitionates of *Cissus adnata* i.e. petroleum ether soluble fraction (PESF), chloroform soluble fraction (CSF), dichloromethane soluble fraction (DCMSF) demonstrated gentle to direct thrombolytic action.

Table 2: Thrombolytic Activity (in terms of % of clot lysis) of leaves of *Cissus adnata*.

Fractions	Weight of empty eppendorf tube	Weight of clot containing eppendorf tube before clot disruption	Weight of clot containing eppendorf tube after clot disruption	% of clot lysis
MESF	4800.9	5819.4	5672.0	14.47
PESF	4816.5	5792.8	5573.8	22.43
DCMSF	4795.4	5675.0	5411.2	29.9
CSF	4876.0	5701.9	5434.4	32.39
AQSF	4674.6	5534.5	5170.6	42.32
Blank	0.919	1.476	1.275	36.09
SK	0.905	1.913	1.24	66.77

W1 = Weight of eppendorf blank; W2 = Weight of clot containing eppendorf; W3 = Weight of clot containing eppendorf after clot disruption; SK =Streptokinase

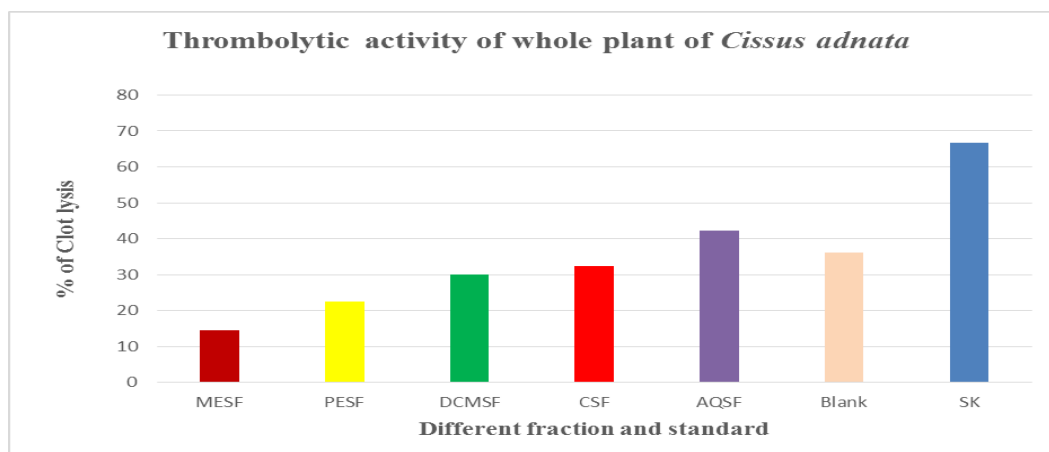


Figure 1: Graphical representation of thrombolytic activity of whole plant of *Cissus adnata*.

From this examination, it can be reasoned that few of the extractives of *Cissus adnata* demonstrated gentle to direct cluster lysis movement. When found these home-grown arrangements might be fused as a thrombolytic specialist for the change of the patients experiencing Atherothrombotic infections. This is just a preparatory review and to make last remark the concentrate ought to altogether examine phytochemically and pharmacologically to misuse their restorative and pharmaceutical possibilities.

Membrane Stabilizing Activity

Heat induced haemolysis

The different concentrates of whole plant of *Cissus adnata* at concentration 1.0 mg/mL were used to evaluate the movement against lysis of human erythrocyte film prompted by warmth, when compared with the standard acetyl salicylic corrosive (0.10 mg/mL) (Table- 1.3). At a grouping of 1.0 mg/mL and in warmth incited condition the petroleum ether soluble fraction portion restrained most potent rate of haemolysis of RBC which is around 51.10% when contrasted with 42.20% hindered by acetyl salicylic corrosive (0.10 mg/mL).

Table 3: Effect of different extractives of leaves of *Cissus adnata* on heat induced haemolysis of erythrocyte membrane.

Sample code	Concentration	% inhibition of haemolysis (Heat induced)
ME	1 mg/mL	67.17
PESF	1 mg/mL	35.1
DCMSF	1 mg/mL	76.7
CSF	1 mg/mL	30.9
AQSF	1 mg/mL	10.12
ASA	0.10 mg/mL	42.20

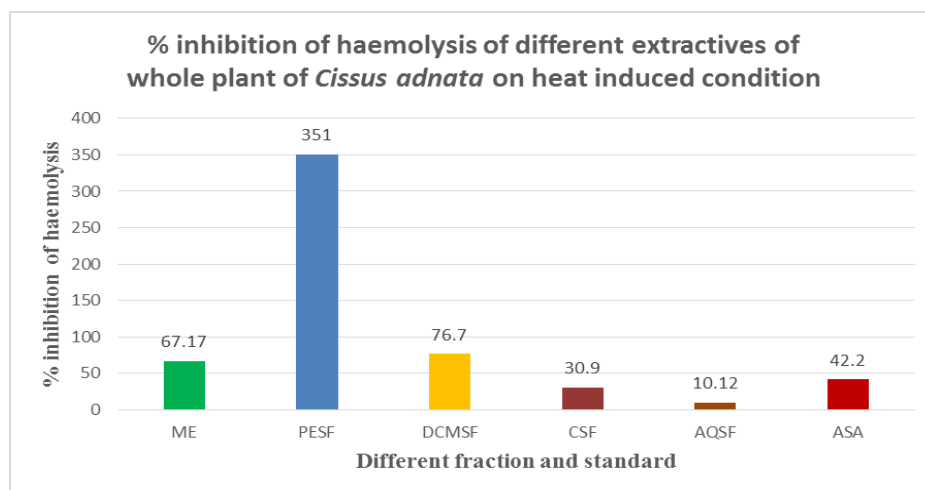


Figure 2: Graphical representation of % inhibition of haemolysis of different extractives of whole plant of *Cissus adnata* on heat induced condition.

In the hypotonic solution of the extract of *Cissus adnata* in several fractions inhibited 67.17% in methanol fraction, 351 in petroleum ether fraction, 76.70 in dichloromethane fraction, 30.9 in chloroform fraction, 10.12 aqueous fraction. These are comparable with the standard acetyl salicylic acid which inhibited 42.20% of hemolysis of RBC. The extract of *Cissus adnata* in several fractions inhibited significantly hemolysis of RBC.

CONCLUSION

All the conducted experiments in the present study are based on crude extract and are considered to be preliminary and more sophisticated research is necessary to reach a concrete conclusion about the findings of the present study. It can be concluded that the extracts in several fractions of the plant *Cissus adnata* can be used to design anti-thrombolytic agent due to its significant thrombolytic activity. The membrane stabilizing experiment indicates as this plant being able to inhibit the hemolysis of RBC to a pretty good extent.

ACKNOWLEDGEMENT

We are greatly thankful to the Department of Pharmacy of BRAC University and Department of Pharmacy of State University Bangladesh for carrying out the analysis and supporting with solvents and materials.

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