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Comparison Estimation of Anticoagulant, Thrombolytic and Fibrinolytic Activity of *Salvia Officinalis* Methanolic and Chloroform Leaves Extract.

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ABSTRACT: *The aim of this study was to investigate the in vitro thrombolytic activity, fibrinolytic, and anticoagulant activity of salvia officinalis leaf methanolic and chloroform extract. Anticoagulant method was done by simple white tile clot observation method.Step by step clot lysis observe distilled water take as negative control. Thrombolytic activity were done by % clot lysis were streptokinase is use as standard and fibrinolytic activity were done by % clot lysis method were urokinase serial dilution used as standard. We concluded that chloroform contain some anticoagulant, thrombolytic and fibrinolytic activity but methanolic not show satisfactory result.*

KEYWORD:Urokinase, Streptokinase, % Clot Lysis

INTRODUCTION

Approximately 80 % of human population used or believed in herbal treatment for different alignments due to fewer side-effects. In the past century, about 121 new pharmaceutical products were formulated thatare based on the traditionally obtainedsources (1). Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs(2). Anticoagulants (antithrombics, fibrinolytic, and thrombolytic) are those drug that work to prevent the coagulation of blood. Fibrin is an insoluble protein involved in blood clotting; the primary type is a normal body process, whereas secondary fibrinolysis is the breakdown of clots due to a medicine, a medical disorder, or some other cause. These anticoagulants are used to treat patients with Deep-Vein Thrombosis (DVT), Pulmonary Embolism (PE) and to prevent emboli in patients with atrial fibrillation (AF) and mechanical prosthetic heart valves (3).salvia officinalis reported that it contain antioxidant activity(4), memory(5), Cancer(6), obesity(7), antibacterial(8), anti-diarrheal and antispasmodic activities(9).

MATERIALS AND METHODS

Plant sample Collection

Fresh plants were collected from the local garden of Bharat and scout guide campus (Bhopal) in the month of May at about two kg plant materials. Material was shade dried until the moisture was removed.The material was grinded and sieved to obtain uniform material .One kg of material was treated with petroleum benzene for two day by maceration method to defatification method and then treated with methanol for five consecutive days by maceration method in aclose container. % yield of extract were obtain

$$\% \text{ yield} = \frac{w \quad (g \quad)o}{w \quad (g \quad)o} \quad t \times 100$$

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Phytochemical estimation

Chloroform and methanolic extract of *salvia officinalis* were done by standard protocol

T**hrombolytic activity of both methanolic and chloroform extract** (10)

Preparation of plant extracts for activity

10mg methanolic or chloroform extract of *salvia officinalis* was suspended in 10ml distilled water. The mixture was shaken vigorously on a vortex for 5-10 min. The mixture was kept aside for 24 hrs at room temperature to make the crude extract soluble in water. The soluble part of extract in water was separated as supernatant and the insoluble part was found as sediment part. The supernatant was then separated using Whatman filter paper. Then this solution is ready for thrombolysis study.

Sampling of blood / Specimen

Healthy volunteers who are not on any type of medication for past 10 days were selected for the study. 4ml of venous blood was withdrawn from each human volunteer and transferred to different pre weighed sterile ependroff tubes (0.5ml/tube). The tubes were now incubated at 37^0C for 45 minutes. After clot formation serum was drawn out without disturbing the clot formed. Each tube having the clot was again weighed to estimate the clot weight.

Clot Weight= Weight of clot formed in the tube-weight of empty Tube

To each tube containing clot with proper labeling was done and 100 µl of methanolic, chloroform extract of was added in each tube gently. To positive control/ standard, tube Streptokinase was added and distilled water was added to negative control. All the tubes were then incubated at 37°C for 90 minutes and observed for clot lysis. After incubation the supernatant fluid released was removed slowly and the tubes were weighed again to observe the difference in weight after clot disruption. Difference between weight taken before and after clot lysis was plot as ratio to obtain the percent of clot lysis

The test was repeated ten times with the blood samples of ten healthy volunteers.

$$\%\mathbf{c} = \frac{\mathbf{w}}{\mathbf{to}} \times \mathbf{100}$$

Fibrinolytic activity of both methanolic and chloroform extract.(11)

The Fibrinolytic activity of salvia officinalis leaves extracts was done by using urokinase (UK) as a standard.

Stock solution of UK in distilled water and prepared the serial dilution (5, 00,000IU, 2, 50,000; 1, 25,000; 62,500 and 31,250 IU dilutions).

Blood collection of human volunteers (10ml)+ add anticoagulant sodium citrate and then centrifuged it at10,000rpm for10 min. collected the supernatant plasma in fresh tubes. Preparation of the plasma clot was done according to the method reported by Pharmacopeia (1960).

Serial dilutions of urokinase with distilled water were taken as a standard, normal saline as blank/control and 1mg/ml concentration of methanolic and chloroform test extract of *salvia officinalis* was taken for experimental study.

Fibrinolytic assay

2ml sterile ependroff tube were taken and weight it before the plasma taken (0.25ml) along with 50ul calcium chloride (1%) solution. Tubes were incubated at 37 ^{0}c for 45 min. After clot formation, the tube was weighed again to determine the clot weight by subtracting the empty weight of the tube that was taken before the addition of the plasma from the weight of the tube with clot

(Clot weight = weight of clot containing tube – weight of empty tube).

Serial dilutions of Urokinase or 1 mg/ml test extract were added to the tube containing clot and incubated at 37 °C for 90 minutes. After incubation, fluid obtained due to clot lysis was completely removed carefully

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from the tubes. The tubes were again weighed to observe the difference in weight after clot lysis. The difference obtained in weight, before and after clot lysis was expressed as percentage of clot lysis.

Anticoagulant activity

Anticoagulant activity of the plant (leaf) extract studied .A clear vein puncture was done; 3ml of blood were drawn and transferred in to 4 Eppendorf tubes containing 0.5ml of blood each. A clean tile was taken; the chloroform, methanolic extract of *salvia officinalis* was taken in tubes. Place a clotted blood on the tile. Add chloroform extract and observed for anticoagulation and place clotted blood on the tile and add methanolic extract of the plant (leaf) and observed for the anticoagulation for 5 to 15 min. Place a 0.5ml of blood in the tile and add distilled water taken as negative control and observed for the anticoagulation.

RESULT

Table 1: Phytochemical estimation of chloroform and methanolic extract.

Test of Carbohydrate	Test	Methanolic extract	Chloroform extract
	Molisch test	-ve	-ve
	Fehling test	-ve	-ve
	Benedict test	-ve	-ve
	Barfoed test	-ve	-ve
Test of proteins and amino acids			
	Biuret test	-ve	-ve
	Ninhydrin test	-ve	-ve
Test of glycosides			
	Legal test	-ve	-ve
	Keller killani test	-ve	-ve
Test of alkaloids			
	Hager test	+ve	+ve
	Wagner test	+ve	+ve
Test of flavonoid			
	Lead acetate test	+ve	+ve
	Alkaline reagent test	+ve	+ve
Test of triterpenoids and steroids			
	Salkowski test	-ve	+ve
	Libermann burchard test	-ve	+ve
Test of tannic and phenolic compounds			
	Ferric chloride test	+ve	+ve
Test of saponins			
	Froth test	-ve	-ve

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Table 2. Table represent the thrombolytic activity of methanolic and chloroform extract.

Sample	% Thrombolytic/% clot lysis
Streptokinase	69.74%±2.162
Methanolic	8.01%±1.223**
Chloroform	16.13%±0.583**

In our table stat 32 software was used to estimate the data comparison with many group ,one way ANOVA followed by bonfirone when compared with streptokinase.p>0.001 shows as **, p> 0.50 as *.

GRAPH 1. % Thrombolytic activity

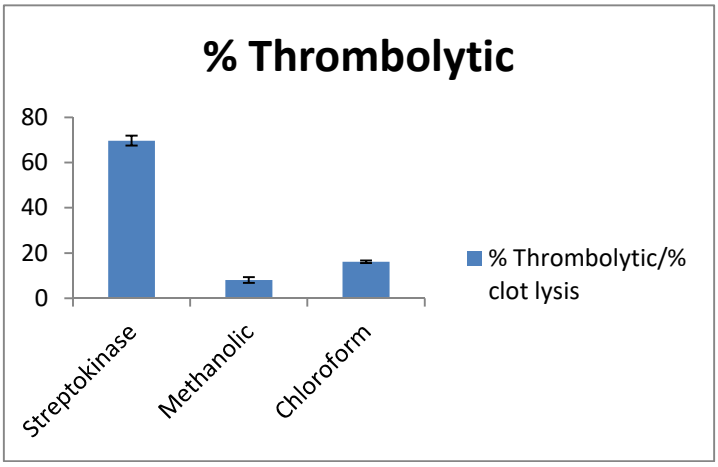

Table 3. Table represent the fibrinolytic activity of methanolic and chloroform extract

Urokinase serial dilution stand curve table

s.no	Urokinase IU	% clot lysis
1	**5,00000**	**100**
2	**250000**	**80.6**
3	**125000**	**70.9**
4	**62500**	**67.7**
5.	**31250**	**66.6**

Graph 2. Percentage clot lysis graph of standard urokinase

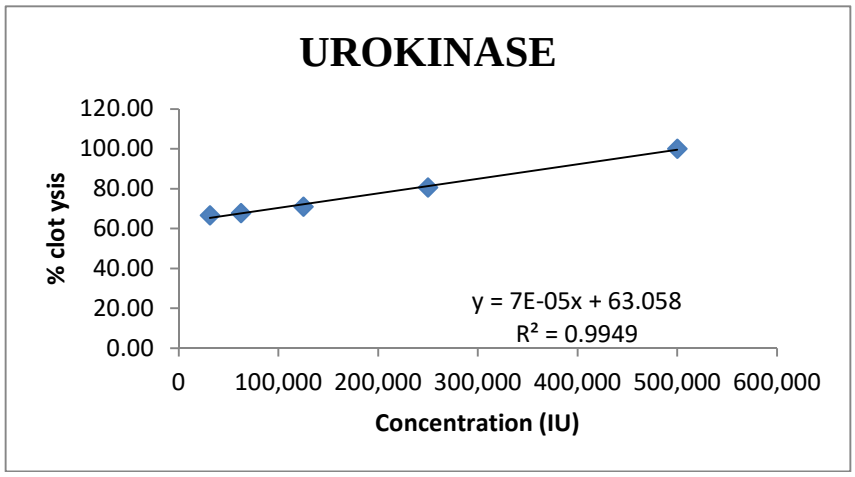

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Table4: Clot lysis at 10 mg/ml concentration of methanol extract of *salvia officinalis*.

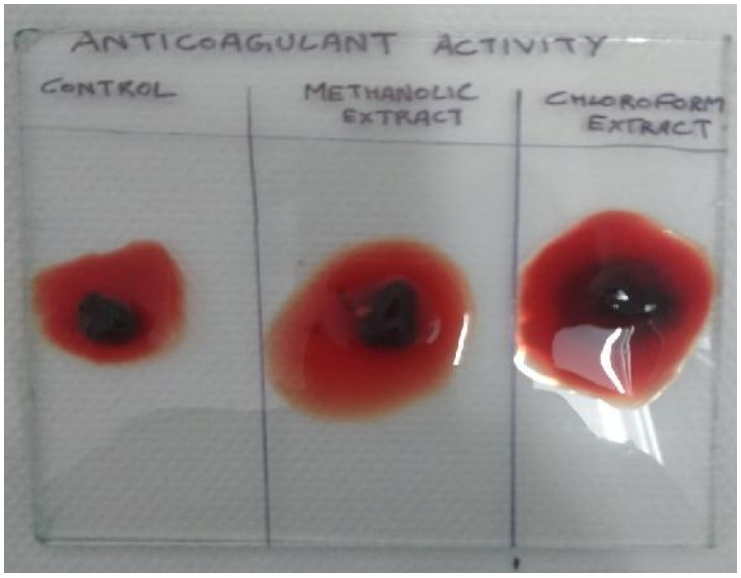
s.no	Methanolic % clot lysis	Chloroform % clot lysis
1	14.12	37.35

Table 5. Table represent the anticoagulant activity of methanolic and chloroform extract

Sample	Anticoagulant activity
Distilled water	-ve
Methanolic extract	+ve
Chloroform	++ ve

Above data represent that –ve sign shows that absent, +ve shows that less , ++ve show good anticoagulant activity.

Picture 1.blot anticoagulant activity of control ,methanolic, chloroform extract.



RESULT AND DISCUSSION

In our present investigative study our plant *salvia officinalis* shrub contains large chemical constituents that were reported in previous study and we also examined through standard phytochemical estimation protocol and it prove that plant containsflavonoids, tannins, alkaloids, steroids etc. and absence of carbohydrates, proteins. Our blood contain many different types of blood clotting meachism and many chemicals participate and conclude the clotting at required place but some time the clotting phenomena causes serious effect on the body like thrombosis in the blood vein and main reason of blockage some time. Coagulation caused on the rightplace is good but some time we do not require coagulation so we use anticoagulant. In our present investigation we observed the comparison between chloroform and methanolic extract of *salvia officinalis* by different parameters like anticoagulant, fibrinolytic and thrombolytic. In table 2 and graph 1 there is % clot lysis of both extracts and standard drug. Standard streptokinase showed best result but as compared to both extract the chloroform extract showed more thrombolytic activity. In table 4, and graph2 represent that when we compared with the standard value chloroform had good fibrinolytic activity as compared to methanolic extract .In anticoagulant activity we directly observed that chloroform extract show more activity as it dissolved more clot as compared to methanol and distilled water. From all the activities we say that our chloroform extract contain good anticoagulant, fibrinolytic and thrombolytic activity but not as such as standard.

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