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Research Article

PHYTOCHEMICAL SCREENING AND *IN VITRO* THROMBOLYTIC ACTIVITY OF *CLERODENDRUM PHLOMIDIS* ROOTS

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ABSTRACT

The aim of the present study is to evaluate the thrombolytic activity of aqueous and ethanol extracts of *Clerodendrum phlomidis* roots. The activity was assessed by thrombolytic assay (clot lysis) using streptokinase as standard. The preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, phenolic compounds like tannins and flavonoids. Aqueous and ethanol extracts showed 48.53 % and 30.13 % clot lysis respectively while the standard (streptokinase) showed 50.74 % clot lysis. Aqueous extract at 20 mg/ml dose level showed significant (***P < 0.001) clot lysis when compared to control. The result indicates that aqueous extract has better thrombolytic activity compared to ethanol extract.

Keywords: *Clerodendrum phlomidis*, Flavonoids, Streptokinase, Tannins, Thrombolytic activity, % clot lysis

INTRODUCTION

Thrombosis is a process of clotting of blood vessels which may cause blockage in blood vessel as a result of haemostasis failure. Thrombolytic agents are commonly used to prevent the formation of thrombus thus preventing life-threatening disorders like myocardial infarction, vein thrombosis, ischemic stroke, arterial thrombosis and pulmonary embolism.[1,2] These thrombolytic agents either directly or indirectly activate plasminogen which is the precursor for plasmin and destroys the fibrin surrounding the clot. But starting from first generation thrombolytic agents like streptokinase, urokinase to recent third generation drugs like reteplase have side effects like severe internal bleeding, bronchospasm, uncontrolled hypertension, hemorrhagic cerebrovascular diseases etc.[3] Hence there is lot of increasing interest in identifying thrombolytic agents of natural origin.

Clerodendrum phlomidis L. f. *(*Verbenaceae*)* is a large shrub distributed throughout drier parts of India especially in sub Himalayan regions and certain parts of South India.[4] It is the accepted botanical source of the Ayurvedic drug Agnimantha.[5,6] *C. phlomidis* is used in the treatment of inflammation, diarrhoea, worm infestation and as demulcent in gonorrhea. The roots are used in the treatment of polyuria, cardiac diseases, oedema and diabetes.[7,8] The plant has been reported for anti-inflammatory[9,10] (leaf, aerial parts, root bark), antidiabetic[11] (stem), hepatoprotective[12] (whole plant), anti-arthritic[13] (leaves), antifungal[14] (leaves and stem), anti fertility[15] (root), immunomodulatory[16] (root) and anti-diarrhoeal [17] (leaves) activity. Several phytoconstituents have been reported in different parts of the plant particularly in roots ß-sitosterol, ceryl alcohol, clerodin, clerosterol and clerodendrin A were reported[18]. Although the root of *C. phlomidis* is traditionally used in the treatment of cardiac diseases, no work has been reported on the thrombolytic activity of roots hence the present study has been undertaken.

MATERIALS AND METHODS

Collection of plant material

The roots of *Clerodendrum phlomidis* was collected from Thuthukudi district of Tamil Nadu in January 2017. The plant material was identified and authenticated by Dr. V. Chelladurai, Taxonomist. Botanical identification was carried out using various floras.[19,20] Herbarium specimen was prepared as per standard methods[21] and a voucher specimen (Mrunal No. 052) was deposited in the Department of Pharmacognosy, Faculty of Pharmacy, M.S.Ramaiah University of Applied Sciences, Bangalore along with the sample of crude drug for future reference. The plant material was thoroughly washed with water to remove the adhering dirt and sandy material, cut into small pieces and shade dried.

Preparation of aqueous and ethanol extracts

The collected root material was washed thoroughly and powdered. Aqueous extract was prepared by macerating the drug with chloroform water for 24 hrs and ethanol extract by continuous hot percolation method (soxhlet apparatus) using 95 % ethanol. The extracts were concentrated to dryness under reduced temperature and pressure.

Preliminary phytochemical screening

Preliminary phytochemical screening of the ethanol and aqueous extracts were done as per standard methods[22, 23].

***In vitro* Thrombolytic assay[24]**

In vitro clot lysis activity of *C. phlomidis roots* was carried out according to the method of Dewan *et al.,* with slight modification.

Preparation of Standard drug Streptokinase (SK)

Commercially available lyophilized S-kinase ™ vial of 1500000 IU was used as standard. 5ml of 0.9 % sodium chloride was added to the vial, mixed thoroughly and this solution was used as a stock from which 100 µl (30,000 IU) was used for *in vitro* thrombolytic assay.

Preparation of test sample (extracts)

The aqueous and ethanol extracts were dissolved in distilled water and ethanol respectively to prepare different concentrations (5, 10, 20 mg/ml respectively) of the test samples. 100 µl of the aqueous and ethanol extracts of the plant was added to the micro-centrifuge tube containing the clots to check thrombolytic activity.

Procedure

Approximately 12 ml of whole blood was withdrawn from healthy rat (Institutional Animal Ethical Committee certificate No: XVIII/ MSRFPH/M-04/8.2.2017). From the 12 ml of blood collected 0.5 ml was transferred to different pre weighed sterile micro-centrifuge tube (0.5 ml/tube). The tubes were then incubated at 37ºC for 45 min. After the formation of the clot, serum was completely removed without disturbing the formed clot and the clot weight was determined.

Clot weight = Weight of tube containing clot – Weight of empty tube

All the micro centrifuge tubes were labelled properly and 100 µl of the aqueous and ethanol extracts were added at various concentration (5, 10 and 20 mg/ml) respectively. 100 µl of 15, 00,000 I.U Streptokinase was used as standard. 100 µl of distilled water and ethanol were added separately to respective control tubes. The tubes were then incubated again at 37ºC for 90 min to observe the clot lysis. After incubation, the fluid present was removed from the tubes and the tubes were re-weighed to determine the difference in weight after clot disruption. The assay was done in triplicates for each sample. The results of thrombolytic assay are expressed as percentage of clot lysis.

% of clot lysis = (Weight of released clot / Clot weight) × 100

Statistical analysis: The data were expressed as mean ± SEM and tested with One Way ANOVA followed by Tukey-Kramer multiple comparison test.

RESULTS AND DISCUSSION

In recent years attention has been focused on the traditional system of medicines for various treatments. Plant based drugs have been used for the treatment, control and prevention of illnesses in humans as they are known to have lesser side effects when compared to synthetic drugs. In the present study the aqueous and ethanolic root extracts of *Clerodendrum phlomidis* showed the presence of carbohydrates, glycosides, phytosterols and phenolic compounds like tannins and flavonoids in preliminary phytochemical screening. In the present study, aqueous extract of *C. phlomidis* root at 20 mg/ml showed extremely significant (***P< 0.001) thrombolytic activity when compared to control. Ethanol extract at 10 and 20 mg/ml showed moderate clot lysis activity. However, the percentage of clot lysis induced by aqueous extract was comparable with the standard Streptokinase. The percentage of thrombolytic effect of aqueous root extract of *Clerodendrum phlomidis* at concentration of 20 mg/ml is 48.53 ± 6.99 % while that of streptokinase is 50.74 ± 2.44 %. Ethanol extract at 10 and 20 mg/ml showed moderate clot lysis activity. Therefore it can be stated that the aqueous extract of root of *Clerodendrum phlomidis* has significant anti-coagulant property.

Table 1: Preliminary Phytochemical screening of aqueous and ethanol extracts of *C. phlomidis* root

Test	Aqueous extract	Ethanol extract
Alkaloids	+	+
Carbohydrates	+	+
Glycosides	+	+
Phytosterols	-	+
Fixed oils and fats	-	-
Tannins	+	+
Flavonoids	+	+
Proteins and amino acids	-	-

+ = Present, - = absent

Table 2: Clot lysis of aqueous and ethanol extract of *C. phlomidis* root

Concentration (mg/ml)	Clot lysis (%) (Aqueous extract)	Clot lysis (%) (Ethanol extract)
Control	8.18 ± 1.01	8.96 ± 2.93
Standard (Streptokinase)	50.74 ± 2.44***	50.74 ± 2.44***
5	28.64 ± 2.78*	22.24 ± 3.57ns
10	31.53 ± 2.23**	27.01 ± 5.17 *
20	48.53 ± 6.99***	30.13 ± 4.30*

Values are expressed as mean ± SEM n=3, ***P< 0.001, **P< 0.01 and *P< 0.05 in comparison with control

CONCLUSION

The result of the present study indicates that the aqueous and ethanolic root extracts of *Clerodendrum phlomidis* possess thrombolytic activity. Aqueous extract was found to have better thrombolytic effect when compared to ethanol extract. Since the plant is showing a promising thrombolytic effect, it can be efficiently used in the treatment of cardiac diseases. A further investigation on the effect of *C. Phlomidis* root extracts in acute myocardial infractions has to be undertaken.

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