# In-Vitro Thrombolytic Activity of Methanolic Extract of Physalis Minima Linn

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*Abstract* - **The present study was designed to investigate the thrombolytic activity of methanolic extract of whole plant of Physalis minima Linn. Phytochemical analysis of the crude extract revealed the presence of steroid(s), alkaloid(s), glycoside(s), phenol, tannin, protein(s), gum and mucilages. Thrombolysis is the breakdown (lysis) of blood clots by pharmacological means. It works by stimulating fibrinolysis by plasmin through infusion of analogs of tissue plasminogen activator (tPA), the protein that normally activates plasmin. These are widely used for the management of myocardial infarction, thromboembolic strokes, deep vein thrombosis and pulmonary embolism. Currently used synthetic drugs causes adverse effects such as major bleeding, cardiac arrhythmias, cerebrovascular hemorrhage and anaphylactic reaction, so there is a need to investigate some more safe natural thrombolytic agents. The methanolic extract was found to have significant thrombolytic activity (49.18±3.41%) compared to the effect of Streptokinase (79.6±1.10%) used as a positive control and water (2.44±0.62%) used as a negative control. Therefore, the study tends to suggest good thrombolytic activity of crude methanolic extract of Physalis minima Linn in-vitro; however, in vivo thrombolytic potentiality and active component(s) of the extract for clot lysis are yet to be discovered.**

*Index Terms* - **Physalis minima, Thrombolytic activity, Streptokinase.**

## INTRODUCTION

Recently Stroke has been severe problem worldwide. Stroke is a time sensitive medical emergency and requires prompt recognition and treatment to reduce morbidity and mortality. It can cause permanent neurological damage or death. A stroke sometimes referred as cerebrovascular accident (CVA), cerebrovascular insult (CVI) or colloquially brain attack. It is the loss of brain function due to a disturbance in the blood supply to the brain. Risk factors for stroke include old age, high blood pressure, previous stroke or transient ischemic attack (TIA), diabetes, high cholesterol, tobacco smoking and arterial fibrillation. high blood pressure is the most important modifiable risk factor of stroke.

There are two type of stroke as shown in figure 1. First ischemic stroke, caused by forming a thrombus (blood clot) that develops in the arteries supplying blood to the brain. This type of stroke is usually seen in older persons, especially those with high cholesterol and atherosclerosis (a buildup of fat and lipids inside the walls of blood vessels) or diabetes. About 87% of all strokes are ischemic strokes. Second hemorrhagic stroke, when a blood vessel bursts, causing bleeding of blood vessels of brain, either directly into the brain parenchyma or into the subarachnoid space surrounding brain tissue. As a result the affected area of the brain cannot function on normally, which might result in an inability to move one or more limbs on one side of the body1.

Treatment of stroke starts with taking drugs that break down clots and prevent others from forming. Emergency procedures include administering TPA directly into an artery in the brain or using a catheter to physically remove the clot. This injection needs to take place within 4.5 hours. Another treatment includes anticoagulation therapy, it is the basis of management, and the proper choice of thrombolytic drugs to decrease platelet aggregation or interfere with the clotting process can be critical. All available thrombolytic drugs have some limitations and adverse effects like first generation drugs (streptokinase and urokinase) show anaphylactic reaction, systemic fibrinolysis, thrombocytopenia, drug-drug interaction, hemorrhage, unpredictable dose response relationship need of intense monitoring, allergic reactions and reocclusion. Immunogenicity is another important issue which restricts the multiple treatments of given patients with streptokinase. This can increased mortality and morbidity associate with thrombotic complications so to overcome these all factors need to

develop new nature origin thrombolytic agents with less risk factor and minimum adverse effects. Herbal medicine is one of the oldest and most universal system of health care system. The novel delivery approach towards the plant based drug is gearing up and success have achieved in making phytoconstituents more bioavailable and stable2.
This research aims to investigate the thrombolytic effect of Physalis minima Linn belongs to the family solanaceae, which is commonly known as wild cape gooseberry in English. The plant is reported as antispasmodic, anti-diabetic, antioxidant, anti-inflammatory, antibacterial, diuretic, laxative, useful in inflammations, supplement for vit.c, enlargement of spleen and abdominal troubles, antilipid peroxidation, hypoglycemic, antitumor. Variant phytochemical constituents were recorded in Physalis minima plant such flavonoid, tannin, phenolic, steroids, alkaloids compounds3.

## MATERIAL AND METHODS

Collection of plant material:
Fresh plant (Physalis minima Linn.) was collected from farm and extracted with 70% ethanol by maceration method at room temperature. Streptokinase purchase from vendor containing 1,50,000 UI.

Preparation of the crude methanolic extract:
The whole plant was shade dried and then grinds into coarse powder with the help of a suitable grinder. The powder was taken in a clean, flat-bottomed amber glass container and soaked in 70% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional stirring. The whole mixture then underwent cotton filtration followed by filtration with whatmann filter paper. The filtrate was kept at room temperature to evaporate the solvent thus crude extract was obtained.

Phytochemical Screening:
Phytochemical studied was carried out for identification of chemical groups present in methanolic extract of Physalis minima Linn. using standard procedure. All the extracts were undergone preliminary phytochemical analysis to identify the nature of secondary metabolites such as alkaloids, carbohydrate, glycosides, saponins, steroids, terpenoids, phenolics, flavonoids and protein present in the plan

Functional groups identification:
The FTIR spectrum was used to identify the functional groups of the active components present in extract based on the peaks values in the region of IR radiation. When the extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio.

Test procedure for in-vitro thrombolytic test:
Animal:
Wistar Albino Rats weighing 200-250g were used in this work. Animals were of mixed sexes. They were housed in clean and dry gauzed cage with free access of food and water.

Preparation of Extract solution for thrombolytic Test:
1g extract was suspended in 10ml distilled water and shaken vigorously over a vortex mixer. Then the suspension was kept overnight and decanted to eliminate the soluble supernatant, which was filtered through filter papers (Whatman No. 1). The solution was then ready for inside vitro evaluation of clot lysis activity.

Preparation of Streptokinase (SK) Solution:
On the commercially available lyophilized SK vial of just one, 1,50,000 I. U., 5 ml clean distilled water was added along with mixed properly. This suspension was used as being a stock from which 100 µl was used for throughout vitro thrombolysis.

Procedure :
Venous blood drawn from healthy rat volunteers was transferred in a number of pre-weighed sterile Epen drop tube (500µl/tube) and incubated at 37 °C for 45 minutes. After clot formation, serum was completely removed (aspirated around without disturbing the clot formed). Each tube having clot was weighed for the clot weight (Clot weight = body weight of clot containing tube - weight of tube alone). After weighing add 100 µl of plant extract in one tube and in another tube add 100 µl of Streptokinase solution and in last tube add water. All the tubes were then incubated at 37 °C for 90 minutes and observed for clot lysis. After incubation, fluid obtained was removed as well as tubes were again weighed to

observe the difference in weight right after clot disruption as shown in figure no 2. Difference obtained throughout weight taken before and right after clot lysis was expressed in percentage4-18.

Thrombolytic activity of methanol extract of Physalis minima Linn. block up lysis. Streptokinase and water were used being a positive and negative (non-thrombolytic) respectively. The experiment was repeated a few times with the blood samples regarding different volunteers.

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

## RESULTS AND DISCUSSION

Phytochemical Screening:

Phytochemical screening conducted on the plant extract revealed the presence of phyto-constituents which explore for medicinal as well as physiological activities. In this research work plant extract exhibited flavonoids, steroids, alkaloids, tannins and phenolic compounds as shown in Table No.1. It could be predicted that these phytochemicals may be responsible for its thrombolytic activity.

In-vitro thrombolytic Activity:

Addition of 100 µl Streptokinase , a positive control (30,000 IU), to the clots and subsequent incubation for 90 minutes at 37 °C, showed 79.6 % lysis of clot as shown in Table no. 2. On the other hand, distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot 2.44 % as shown in Table no. 2. The mean difference of in percentage of clot lysis between positive and negative control as shown in figure no 3 was found to be statistically significant. In this study methanolic extract of Physalis minima Linn show significant thrombolytic activity 52.22% as shown in Table no. 2 and 3.

## CONCLUSION

From the above study it can be concluded that the methanol extract of Physalis minima Linn. Possess a significant thrombolytic activity. So, further comprehensive pharmacological and phytochemical investigations are needed to elucidate the specific chemical compounds responsible for thrombolytic activities and their mode of actions. Formulation development is also for future scope.

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Table No. 1: Showing presence of phytoconstituents in methanol extract of Physalis minima Linn.

| Tested groups | Methanolic extract of *Physalis minima* Linn. |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Steroids | + |
| Tannin | + |
| Phenolic compounds | + |
| Terpenoids | + |
| Glycosides | - |
| Saponins | - |

Table No. 2 : Thrombolytic Activity of methanol extract of Physalis minima Linn.

| SAMPLE | % OF CLOT LYSIS |
|---|---|
| Streptokinase | 79.6 % |
| *Physalis minima* methanol extract | 52.22 % |
| Water | 2.44 % |

Table No.3 : Results of clot lysis of methanol extract of Physalis minima Linn.

| Sr. No of Volunteers | Weight of tube | Weight of tube with clot | Weight of tube with clot after lysis | Weight of clot | Weight of lysis | % of lysis | Average % of lysis |
|---|---|---|---|---|---|---|---|
| 1. | 1.106 | 1.690 | 1.408 | 0.584 | 0.282 | 48.28 | 52.22% |
| 2. | 1.092 | 1.605 | 1.321 | 0.510 | 0.284 | 55.36 | |
| 3. | 1.093 | 1.553 | 1.272 | 0.361 | 0.281 | 77.83 | |
| 4. | 1.097 | 1.533 | 1.309 | 0.436 | 0.224 | 51.37 | |
| 5. | 1.096 | 1.599 | 1.428 | 0.403 | 0.172 | 42.54 | |
| 6. | 1.090 | 1.548 | 1.409 | 0.359 | 0.139 | 38.98 | |
| 7. | 1.093 | 1.657 | 1.639 | 0.564 | 0.018 | 32.60 | |
| 8. | 1.106 | 1.598 | 1.370 | 0.322 | 0.228 | 70.80 | |

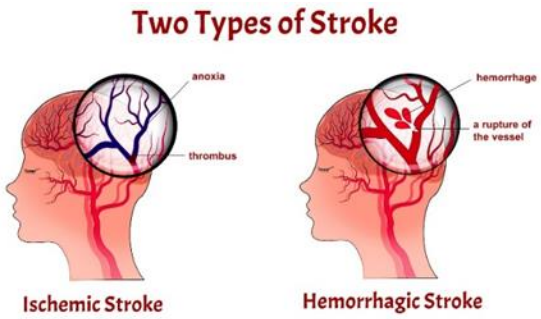


Figure No.1: Types of stroke

(a)Blood clot befor lysis (b) Blood clot after lysis

Figure No. 2: Representation of blood clot before and after lysis.

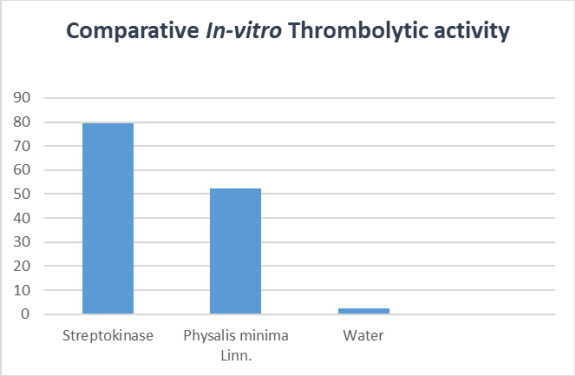


Figure No.3 : Comparative thrombolytic activity by water, Streptokinase and Methanol extract of Physalis minima Linn