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Clot Lysis Activity of Methanolic *Physalis micrantha* Link. Extract

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Abstract

The present study aimed to investigate the clot lysis activity of *Physalis micrantha* and suggests that its methanolic extract has a significant clot lysis effect on clotted human blood in comparison to the control group. The extract produced a concentration-dependent clot lysis effect on the test system. The half-maximal effective concentration (EC_{50}) of the extract is 100.03 ± 2.18 µg/mL. *Physalis micrantha* might be one of the sources of anti-thrombolytic agents. More research is required to isolate and quantify the amount of clot lysis chemical constituents found in this plant.

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Keywords

Physalis micrantha, human blood, clot lysis assay

Introduction

Thrombosis results in thousands of deaths each year (Hunt, 2008). Therefore, it is one of the major global burdens. Thrombous formation in the portal vein affects the hepatic portal vein. Thus, this phenomenon can lead to portal hypertension, reduction of the blood supply to the liver, stroke to the heart, anoxia, and so on (Bekker *et al.*, 2009). Cell death may occur due to a complete deprivation of oxygen and infarction development processes. Thrombosis is also linked to pancreatitis, cirrhosis, diverticulitis, cholangiocarcinoma, and even death in humans. A number of crude biologicals and their derivatives having antithrombotic activity have been reported to date. For example, some oral anticoagulants have been derived from coumarins, which are found in many plants and can be used to treat patients with deep-vein thrombosis, pulmonary embolism, atrial fibrillation, and mechanical prosthetic heart valves (Ansell *et al.*, 2008). Thus, medicinal plants offer hope for treating thrombosis in humans.

Traditionally, *Physalis micrantha* (Family: Solanaceae) is a bitter tonic, appetizing, diuretic, laxative, anti-inflammatoric, antigonorrhoeic (Sinha and Ray, 1988; Yusuf *et al.*, 1994; Joy *et al.*, 1998), and is used for skin infections, diabetes, enlargement of the spleen, and abdominal troubles (Rahman *et al.*, 2010; Hasan *et al.*, 2014), stomach pain, constipation, ear disorders (Parkash and Aggarwat, 2010), cancer (Zakaria and Mohd, 2010), and fever (Rajakaruna *et al.*, 2002). Studies suggest that *P. micrantha* possesses flavonoids, tannins, reducing

sugar, and alkaloids and shows strong DPPH radical scavenging ability (Laryea and Borquaye, 2021), antigonorrheal (Caceres *et al.*, 1995), anti-inflammatory, antipyretic, analgesic (Khan *et al.*, 2009), antibacterial (Ahsan *et al.*, 2009), antiulcer (Gupta *et al.*, 2010), and cytotoxic activity (Leong *et al.*, 2010) properties. This study aimed at evaluation of anti-atherothrombosis activity using an *ex vivo* clot lysis assay for *P. micrantha* demonstrates that the methanolic whole plant extract has a concentration-dependent moderate clot lysis effect on human clotted blood.

Materials and Methods

Plant collection and identification

For this study, *P. micrantha* was collected from the hills of the Forest Research Institute; Chittagong and Rangunia; Chittagong, Bangladesh in October 2014 at day time and was identified by the Forest Research Institute; Chittagong, Bangladesh.

Extraction

The collected plant parts were separated from undesirable materials or plants or plant parts and sun dried at 35 to 50 °C and ground into a coarse powder with the help of a suitable grinder. The powdered material (150 gm powder) was subjected to hot extraction with 97.7% methanol (800 mL) using a Soxhlet Apparatus (Quickfit, England). The obtained extract was collected, filtered, and made to evaporate the solvent below 50 °C.

Reagents and chemicals

Streptokinase (Powder for reconstitution: 30,000 IU) was purchased as DURAKINASE Inj. from Dongkook Pharmaceutical Ltd., Korea.

Clot lysis test (*in vitro*)

Preparation of test sample and controls

The required amount of methanolic extract of *Physalis micrantha* (MEPM) was suspended in double distilled water (DDW) to prepare the highest concentration (80 µg/mL) required for this study, and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant. It was then diluted with DDW to get the following dilutions: 5, 10, 20, and 40 µg/mL. Streptokinase (SK, standard) was reconstituted with water for injection (WFI), while DDW served as vehicle or control group.

Study design

This study was performed with a slight modification of Prasad *et al.*, (2006). For briefly, 4 mL of venous blood drawn from five healthy volunteers was distributed into three different groups: Gr-I (Vehicle), Gr-II (SK) and Gr-III (MEPM). Pre-weighed sterile microcentrifuge tubes marked "Pre-weighed" were used for this purpose. Each tube contained 0.5 mL of blood and was incubated for 45 minutes at 37 °C. After clot formation, serum was completely removed carefully without disturbing the clot. Each tube was weighed again to determine the clot weight (clot weight = weight of clot containing tube – weight of empty tube). 100 µL (5, 10, 20, 40, or 80 µg/mL) of MEPM was added to the clotted blood.

Similarly, 100 µL of SK and 100 µL of DDW were separately added to the standard and control marked tubes. The tubes were then re-incubated at 37 °C for 90 minutes while clot lysis was monitored. After the incubation period, fluid released by each clot was removed carefully and weighed again to determine the lysis of the clot by the action of the test sample or controls. The difference obtained in weight obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis (mean ± SD) by using the following equation:

$$\text{Clot lysis } (\%) = \frac{\text{Weight of lysis}}{\text{Weight of clot before lysis}} \times 100$$

The experiment was duplicated on different days with the same blood donors (both male and female who have not used contraceptives and anticoagulants in the last few months).

Statistical analysis

Values are expressed as mean ± standard deviation (SD). One-way ANOVA (analysis of variance) followed by *t*-student's test by using Graph Pad Prism software (version: 6.0) at a 95% confidence interval considering $p<0.05$.

Results and Discussion

Since human civilization began, we have been dependent on plants for the treatment of almost all kinds of

diseases. A plant having medicinal value is a medicinal plant. In this context, each plant is a medicinal plant because every plant contains thousands of phytoconostients. Nowadays, phytopharmacological investigation has proved that plant-derived chemicals are safe and effective in the remedial of various diseases, including cardiovascular diseases. It is estimated that about 30% of pharmaceuticals are prepared from plant derivatives (Leta *et al.*, 2002). Plant parts, including leaves, stems, fruits, and seeds, have been studied for their anti-coagulant, anti-platelet, and fibrinolytic activity and it has been found that consuming such foods leads to the prevention of coronary events and stroke (Emran *et al.*, 2015). In our other study, MEPM revealed the presence of several important phytochemical groups (including glycosides and alkaloids) which might be responsible for its biological effects. In our present study, the control group (DDW) showed negligible clot lysis (1.07 ± 0.87%) activity. Both standard, SK and test sample MEPM showed significant clot lysis effects on the test system.

The MEPM showed a concentration-dependent clot lysis effect on the human clotted blood. At 5 µg/mL the MEPM produced percentage clot lysis by 6.15 ± 1.23, while at 80 µg/mL it produced 43.27 ± 1.21. The SK at 100 µL (30,000 IU) showed percentage clot lysis of 84.45 ± 0.78. The EC_{50} calculated for MEPM was 100.03 ± 2.18 µg/mL (Table 1). The maximum clot lysis activity observed in the MEPM group means its phytoconostituents are mainly responsible for the thrombolytic activity. This finding indicates the possibility of developing novel thrombolytic compounds from this medicinal plant.

Table.1 Lysis of clots by the test sample and control groups

Treatment groups		Percentage clot lysis	EC_{50} [CI; R^2]
DDW (100 µL)		1.07 ± 0.87	-
SK (100 µL)		84.45 ± 0.78*	-
MEPM (µg/mL)	5	6.15 ± 1.23*	100.03 ± 2.18 µg/mL [94.08 – 124.41 µg/mL; 0.87]
	10	9.16 ± 1.19*	
	20	12.13 ± 1.08*	
	40	28.07 ± 1.39*	
	80	43.27 ± 1.21*	

Values are mean ± SD (standard deviation) (n = 5); One-way ANOVA (analysis of varience) followed by *t*-student's test; *p<0.05 when compared to the DDW (double distilled water: control (vehicle) group); SK: Streptokinase; MEPM: Methanol extract of *Physalis micrantha*; EC_{50}: Half-maximal effective concentration; CI: Confidence of interval; R^2: Co-efficient of determination

In the test tubes, MEPM showed a concentration-dependent clot lysis effect on human clotted blood. In comparison to the control group, it produced a significant clot lysis effect. *P. micrantha* might be one of the sources of thrombolytic agents and will be helpful for the management of thrombosis and improvement of patients suffering from atherothrombotic diseases.

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