

Original Research Article
Pharmacy

***In Vitro* Evaluation of Thrombolytic Potential of Leaf Extracts of *Cleome rutidosperma* DC. (Family: Capparidaceae) and *Pouzolzia zeylanica* (L.) Benn. (Family: Urticaceae) Grown in Bangladesh**

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 DOI: <https://doi.org/10.36348/sjmps.2025.v11i03.014>

| Received: 18.02.2025 | Accepted: 26.03.2025 | Published: 29.03.2025

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Abstract

Cleome rutidosperma DC. (commonly known as Nil Hurbure) and *Pouzolzia zeylanica* (L.) Benn. (locally referred to as Kulluruki) are two medicinal plants widely found in various regions of Bangladesh. *Cleome rutidosperma*, a low-growing herb of the Capparidaceae family, typically reaches a height of up to 70 cm and is found in areas such as Chittagong. *Pouzolzia zeylanica*, a perennial herb from the Urticaceae family, is widely distributed across Bangladesh, particularly in tribal regions like Bandarban, Chattogram, Cox's Bazar, Khagrachhari, and Rangamati. Both plants have been used in traditional herbal medicine for their wide range of pharmacological benefits in treating various diseases. In this study, the methanolic extracts of powdered leaves of *Cleome rutidosperma* and *Pouzolzia zeylanica* were tested for their thrombolytic potential using human erythrocytes. The results revealed that *Cleome rutidosperma* exhibited a clot lysis of $22.96 \pm 2.12\%$, while *Pouzolzia zeylanica* showed $36.15 \pm 1.51\%$ clot lysis. Whereas, the sterile distilled water (negative control) and streptokinase (positive control) demonstrated clot lysis of $4.9 \pm 0.59\%$ and $81.67 \pm 2.62\%$, respectively. The observed thrombolytic activity may be attributed to the presence of phytochemical compounds, such as tannins, alkaloids, and saponins, in the plant extracts. Although no previous research has specifically examined the thrombolytic potential of *Cleome rutidosperma* and *Pouzolzia zeylanica*, our findings suggest that the clot lysis effect of both plants is noteworthy when compared to the positive control and negative control. Based on these results, we propose that the methanolic leaf extracts of *Cleome rutidosperma* and *Pouzolzia zeylanica* possess moderate thrombolytic potential, making them promising candidates for future drug development.

Keywords: *Cleome rutidosperma*, *Pouzolzia zeylanica*, Thrombolytic Activity, Clot Lysis, Phytochemicals, Bangladesh.

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INTRODUCTION

Medicinal plants have long been considered gifts from nature, with evidence indicating their use throughout history. According to the World Health Organization, plants have the unique ability to synthesize a diverse range of chemical compounds that are essential for the treatment of various diseases (WHO, 2004). Herbal medicine has been used since ancient times to address a wide variety of illness (Tandon & Gupta, 2004). These remedies were crafted through the therapeutic expertise of generations of physicians who practiced for centuries (Sheetal & Ashlesha, 2011). Nature has provided us with the invaluable gift of medicinal plants, which are used to treat and prevent diseases (Kalaria Pankti *et al.*, 2012). In Bangladesh, herbal medicine is widely used in

traditional healthcare systems, such as ayurveda, unani, hekimi, and other folk treatments (Ghani, 2006). It is estimated that nearly 80% of the rural population dependent on medicinal plants for primary healthcare. Today, medicinal plants are also becoming increasingly significant in the pharmaceutical industry, particularly for the development of new phytomedicines (Sule *et al.*, 2010).

Thrombosis refers to the formation of blood clots inside a vein or blood vessel, which impedes normal blood flow through the animal body's circulatory system. When a blood artery is damaged, the body generates red blood cells at the site of injury, using platelets (thrombocytes) and fibrin to stop bleeding (Labu *et al.*, 2015). Thrombolysis is the

process of dissolving blood clots through pharmacological means. Injecting tissue plasminogen activator (TPA), the protein which typically activates plasmin stimulates fibrinolysis by plasmin (Wardlaw *et al.*, 2014). Thrombolytic drugs are approved for the short-term treatment of heart attacks and strokes (Chowdhury *et al.*, 2011). Tissue plasminogen activator (TPA) is the most commonly used thrombolytic agent, although other medications can serve the same purpose. Thromboembolic diseases, such as deep vein thrombosis, heart attacks, strokes, and pulmonary embolism, are leading causes of illness and death in both developed and developing countries (Chowdhury *et al.*, 2011; Umesh *et al.*, 2014). As a result, considerable research has been focused on identifying natural compounds from plants that exhibit antiplatelet, anticoagulant, antithrombotic, and thrombolytic properties.

Cleome rutidosperma DC., commonly known as the "fringed spider flower" or "purple cleome," is a species of flowering plant in the Capparidaceae family. This plant is native to tropical and subtropical regions of Asia and Africa (Rojas-Sandoval & Acevedo-Rodríguez, 2022). It typically grows as a low herb, reaching heights of up to 70 cm, and thrives in waste herb, grassy grounds, and similar habitats. The plant features trifoliate leaves and small violet-blue flowers that turn pink in West Africa, though it has also become widespread in various parts of tropical America and Southeast Asia. Different parts of the *Cleome* genus are used for various medicinal purposes, including as a stimulant, anticorbutic, antihelminthic, vesicant, rubefacient, and carminative (Kiritikar & Basu, 1991). Studies have reported its antiplasmodial, locomotor, analgesic, antimicrobial, diuretic, and laxative properties (Bidla *et al.*, 2004). Additionally, the roots of *C. rutidosperma* have reported hypoglycemic effects (Mondal *et al.*, 2009). While the antimicrobial, antioxidant, and anticancer activities of *Cleome* species have been well studied, there have been fewer investigations focused on *C. rutidosperma*. Its bioactive compounds, including phenolics, alkaloids, tannins, terpenoids, and saponins, are believed to contribute to its medicinal properties (Akinsola & Oluwafemi, 2022; Ghosh *et al.*, 2019).

Pouzolzia zeylanica (L.) Benn. (Family: Urticaceae) is a perennial herb that can be either erect or prostrate, with glabrous stems and alternate, membranous leaves. The plant produces minute flowers and small achenes and is commonly found growing along roadsides and in waste areas throughout Bangladesh. Locally known as Kulluruki (Ghani, 2003), this species is widely distributed in both Asian and Australian regions, particularly growing during the monsoon season. *Pouzolzia zeylanica* is traditionally used in various folkloric medicinal preparations (Chutia *et al.*, 2021). It is considered effective for treating conditions such as diarrhea, indigestion, infantile

malnutrition, urinary difficulties, and injuries resulting from falls. Additionally, it is particularly beneficial in managing acute mastitis and pyogenic infections (Tsao & Deng, 2004; Hayet *et al.*, 2008). The plant's leaves have anthelmintic and vulnerary properties, often being used as a cicatrizant for gangrenous ulcers and in the treatment of syphilis and gonorrhea. Leaf juice is also used as galactagogue. Poultices made from the herb are applied to sores, boils, and to alleviate stomachaches (Yusuf *et al.*, 2009). Moreover, this plant contains flavones, flavonoids, tannin, carotene, carotenoids, ascorbic, tartaric, malic and pectic acids, gum, minerals and their salts (Ghani, 2003).

However, no thrombolytic potentials study has been conducted on two medicinal plants of *Cleome rutidosperma* and *Pouzolzia zeylanica*. Hence, the present study was designed to investigate the thrombolytic activities of the leaf extracts of *Cleome rutidosperma* and *Pouzolzia zeylanica* plants.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The plant *Cleome rutidosperma* was collected from Uttarkhan, Uttara, Dhaka in September 2024. It was officially identified by an expert taxonomist Professor Mohammad Zashim Uddin, Professor, Department of Botany, University of Dhaka. An accession number DUSH- 10836 was provided after identification of the submitted voucher specimen of the plant. The specimen has been preserved there for future references. The plant *Pouzolzia zeylanica* was also selected for the study. The plant was collected from Uttarkhan, Uttara, Dhaka, Bangladesh in November, 2023. The collected plant was identified and authenticated by experts in Bangladesh National Herbarium (BNH), Mirpur, Dhaka. An accession number DACB -91733 was provided by BNH after identification of the submitted voucher specimen of the plant. The specimen has been deposited there for future references.

Drying, Grinding and Extraction

The leaf of *Cleome rutidosperma* and *Pouzolzia zeylanica* were collected from the plants for the current study. The freshly collected leaf were immediately cleaned with clean water to get rid of any dirt or undesired items. Furthermore, leaves had undergone to sun-dried for 7 days. When the drying was complete, with the help of high capacity grinding machine the entire leaves were pulverized into a coarse powder. It was then stored in separate airtight container for further use. The extraction of both the plants powder materials of *Cleome rutidosperma* and *Pouzolzia zeylanica* were done by cold extraction process with methanol. Powder materials of *Cleome rutidosperma* DC (280 g) and *Pouzolzia zeylanica* (350 g) had been macerated with 2.5 L and 3.0 L of methanol, respectively in a closed amber container for 10 days with occasional stirring. A new cotton plug and

Whatman filter paper No. 1 were used to filter the solvent mixtures. The consequent filtrate was then concentrated with the rotary evaporator at 35°C and under low pressure to generate a viscous crude extract (11.2 g and 15 g, respectively).

Evaluation of Thrombolytic Activity

The *in vitro* thrombolytic activity of the methanolic leaf extracts of *Cleome rutidosperma* and *Pouzolzia zeylanica* was evaluated using human blood, employing a slightly modified method from Prasad *et al.*, (2007). Streptokinase was used as the standard.

Plant Sample Preparation

The crude extracts of both plants were each suspended in 10 ml of distilled water and vigorously shaken using a vortex mixer. The suspensions were then left overnight, allowing the soluble supernatants to separate. Afterward, the supernatants were decanted and filtered through filter paper. The resulting solutions were prepared for *in vitro* evaluation of clot lysis activity.

Preparation of Streptokinase (SK)

Approximately 5 mL of sterile distilled water was added to the commercially available lyophilized SK vial containing 1,500,000 I.U (Incepta Pharmaceuticals Ltd.) and mixed thoroughly. This resulting suspension was used as the stock solution, from which 100 µL (30,000 I.U.) was applied for the *in vitro* thrombolysis study.

Blood Specimen Preparation

A sample of whole blood (5 ml) was collected from healthy human volunteers with no prior history of oral contraceptive or anticoagulant therapy (the study protocol and volunteer's consent form approved (SUB/SF/EC-2411/14) by the Institutional Ethical Review Committee of the Faculty of Science, Stamford University Bangladesh). 1 ml of this blood was then transferred into pre-weighted sterile Eppendorf tubes and allowed to clot formation.

Determination of Thrombolytic Activity

The Eppendorf tubes were incubated at 37 °C for 45 minutes to allow clot formation. After clot formation, the serum was carefully removed without disturbing the clot, and the weight of each Eppendorf tube containing the clot was measured again (clot weight = weight of tube with clot - weight of the empty

tube). To each Eppendorf tube containing the pre-weighed clot, 100 µl of aqueous solutions of different extracts, along with the crude extract, were added separately. For the positive control, 100 µl of streptokinase was added, and for the negative control, 100 µl of distilled water was used. All tubes were incubated at 37 °C for 90 minutes and monitored for clot lysis. After the incubation period, the released fluid was discarded, and the Eppendorf tubes were reweighed to assess the difference weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis is shown below:

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

Statistical Analysis

All the assays were done in three replicates of each sample and values were taken as mean \pm standard deviation by statistical analysis. Microsoft excel 2010 were used to calculate the experimental results.

RESULTS

This study evaluated the thrombolytic activities of methanolic leaf extracts from two medicinal plants, *Cleome rutidosperma* and *Pouzolzia zeylanica*. A 100 µl aliquot of each plant extract, along with standard streptokinase and sterile distilled water, was added to blood clots, which were incubated at 37°C for 90 minutes. After the incubation period, the results showed that the highest clot lysis (81.67 \pm 2.62%) was achieved by streptokinase, the positive control, while sterile distilled water (negative control) resulted in minimal clot lysis (4.9 \pm 0.59%). The *in vitro* thrombolytic activity revealed that the methanolic leaf extracts of *Cleome rutidosperma* and *Pouzolzia zeylanica* induced clot lysis of 22.96 \pm 2.12% and 36.15 \pm 1.51%, respectively. These results suggest that the clot lysis effect of both plants were moderate when compared to the controls. Statistical representation of the effective clot lysis percentage by sterile distilled water (negative control), streptokinase (positive control), and the two plants extracts are presented in Table 1. Percent clot lysis obtained after treating clots with these two methanolic leaf extracts and appropriate control is shown with a graphical representation in Figure 1.

Table 1: Effect of *Cleome rutidosperma* and *Pouzolzia zeylanica* plants extracts on *in vitro* clot lysis

Extracts/Drugs	Mean \pm S.D. (% Clot Lysis)
Sterile distilled water	4.9 \pm 0.59%
Streptokinase	81.67 \pm 2.62%
<i>Cleome rutidosperma</i>	22.96 \pm 2.12%
<i>Pouzolzia zeylanica</i>	36.15 \pm 1.51%

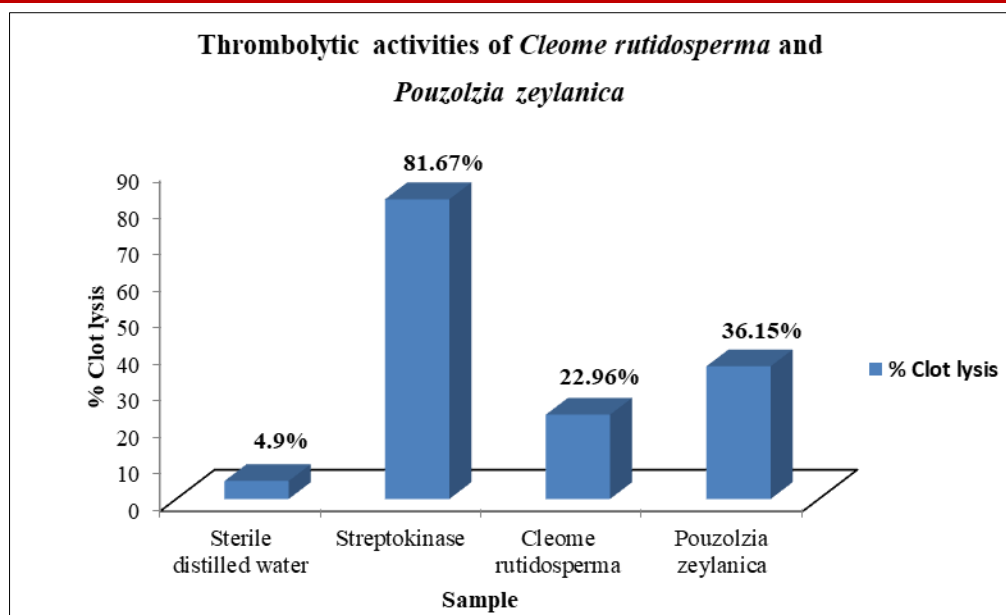


Figure 1: Effect of *Cleome rutidosperma* and *Pouzolzia zeylanica* plants extracts on *in vitro* clot lysis

DISCUSSION

Thrombolytic or fibrinolytic agents are commonly used to treat conditions like cerebral venous sinus thrombosis (CVST) and myocardial infarction. Medications such as tissue plasminogen activator (t-PA), streptokinase, and urokinase are among the most frequently used, but they are associated with significant risks of bleeding and along with high treatment costs (Katzung, 2008).

Platelets, tissue factor, and fibrin play key roles in thrombosis, or blood clot formation, by blocking the surface of endothelial cells and damage to blood vessels (Furie & Furie, 2008). Activated platelets interact with one another active platelets, initiating the thrombotic process. When leukocytes and activated platelets bind together, a complex cascade leads to plaque formation and development is triggered (Das *et al.*, 2013). Thrombolytic treatments, such as fibrinolytic medications, break down the fibrin and fibrinogen present in blood clots to dissolve them. Streptokinase is commonly used to dislodge blood clots by converting excess plasminogen into plasmin (Ramjan *et al.*, 2014). A number of studies have been undertaken by many researchers to identify new herbal sources and natural food supplements that have antithrombotic properties, aiming to minimize side effects (Dewan & Das, 2013).

We also explored the potential clot lysis activity of *Cleome rutidosperma* and *Pouzolzia zeylanica* methanolic leaf extracts. In this investigation, we observed minimal clot disintegration when water was added to the clot, which was consistent with the comparison between the positive control (streptokinase) and the negative control (distilled water). However, when compared to the control group, the methanolic leaf extracts of *Cleome rutidosperma* and *Pouzolzia zeylanica* demonstrated moderate clot lysis activity (as

shown in Table 1 and Figure 1). Notably, there is no previous research work reported on the thrombolytic activity of *Cleome rutidosperma* and *Pouzolzia zeylanica*. Several studies have linked the presence of secondary metabolites, such as tannins, alkaloids, and saponins, to thrombolytic activity (Ramjan *et al.*, 2014; Bhowmick *et al.*, 2014; Ali *et al.*, 2013).

The literature review of the phytochemical screening studies revealed that the methanolic leaf extract of *C. rutidosperma* contains a wide range of phytochemical groups, including alkaloids, flavonoids, tannins, steroids, proteins, saponins, polyphenols, and terpenoids (Prabha *et al.*, 2017). Similarly, the methanolic leaf extract of *Pouzolzia zeylanica* showed the presence of key phytochemical constituents such as carbohydrates, alkaloids, tannins, gums, flavonoids, and saponins as major active components (Jamoh *et al.*, 2018). The thrombolytic activity of both medicinal plants may be attributed to the presence of saponins, tannins, and alkaloids in their methanolic leaf extracts. These leaf of *C. rutidosperma* are commonly consumed as vegetables or added to soups, offering a bitter taste similar to mustard. The leaf sap is used to treat earaches, irritated skin, prickly heat, and convulsions. An extensive review of the literature indicates that the *C. rutidosperma* plant exhibit various biological activities, including antibacterial, wound healing, antioxidant, antidiabetic, diuretic, and laxative effects (Ghosh *et al.*, 2019). In addition to its ethnomedicinal value, *C. rutidosperma* has been widely studied for its bioactive properties. Research has shown that *C. rutidosperma* possesses anti-inflammatory, antinociceptive, antioxidant, antitumor, and antimicrobial activities (Ghosh *et al.*, 2019). The aerial parts of *P. zeylanica* have been used in traditional folk medicine for over 2000 years in Fujian Province for the treatment of skin and soft tissue infections, including

abscesses, gangrenous ulcers, dysentery, syphilis, sores, boils, and gonorrhea (Dangol & Gurung, 1991; Van Sam *et al.*, 2008). Moreover, it has been reported that the methanol extracts of *P. zeylanica* had antioxidant, anti-inflammatory, cytotoxic, antimicrobial potential and analgesic activities (Hossain *et al.*, 2016).

CONCLUSION

In conclusion, the *Cleome rutidosperma* and *Pouzolzia zeylanica* plants extracts demonstrated moderate thrombolytic potentials. Further study is necessary to distinguish and characterize the chemicals that oversee these pharmacological effects. Finally, a rigorous investigation is needed for future drug development to counteract diseases like blood clotting using the leaves of *Cleome rutidosperma* and *Pouzolzia zeylanica*.

Acknowledgement

The author would like to thank to the Department of Pharmacy, Stamford University Bangladesh, Dhaka for providing all the necessary research facilities to carry out the research work.

Conflicts of Interest: The author declares no conflict of interest with this study.

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