

Thrombolytic Activity Test of Spider Plant Leaf Extract (*Chlorophytum Spp.*)

Siska Zafrida¹, Hartini², Ignatius Yulianto³, Melsa kharunnisa⁴

¹⁾ D4 Teknologi Laboratorium Medis, Akademi Kesehatan John Paul II Pekanbaru, Indonesia.
^{2,3,4)} D3 Analisis Kesehatan, Akademi Kesehatan John Paul II Pekanbaru, Indonesia.

Email:

Siskazafrida@akip2.ac.id

ABSTRACT

Cardiovascular disease (CVD) is a disorder of the heart and blood vessels and remains the leading cause of death worldwide. Atherosclerosis and thrombosis are major contributors to CVD. Thrombolytic agents such as urokinase, streptokinase, and nattokinase are effective in dissolving blood clots; however, their prolonged use may cause adverse effects and high treatment costs. Therefore, the development of safer and more affordable natural thrombolytic agents is necessary. This study aimed to evaluate the in vitro thrombolytic activity of ethanol extract from spider plant leaves (*Chlorophytum spp.*). The study employed a post-test only control group experimental design using extract concentrations of 0.5%, 1%, and 2%, with nattokinase as the positive control and distilled water as the negative control. Phytochemical screening revealed the presence of flavonoids, saponins, and tannins in the extract. The thrombolytic activity increased with increasing extract concentration. The ethanol extract demonstrated clot lysis percentages of 2.8%, 4.2%, and 5.5% at concentrations of 0.5%, 1%, and 2%, respectively. Although its thrombolytic activity was lower than that of the positive control, the extract showed concentration-dependent thrombolytic potential. These findings suggest that spider plant (*Chlorophytum spp.*) leaves possess bioactive compounds with thrombolytic properties and may serve as a promising natural source for the development of alternative thrombolytic agents. Further studies, including isolation of active compounds and in vivo evaluation, are recommended.

Keywords: *Chlorophytum spp.*; Thrombolytic activity; Cardiovascular disease; Natural extract; Thrombosis

Introduction

Cardiovascular Disease (CVD) represents the primary cause of mortality worldwide and remains a significant global health challenge today. A critical mechanism underlying the occurrence of cardiovascular disease is thrombosis, characterized by the formation of blood clots within blood vessels that can obstruct blood flow to vital organs, such as the heart and brain. This condition plays a pivotal role in the pathogenesis of coronary heart disease, stroke, and other vascular disorders, necessitating effective therapy to lyse blood clots. This condition plays a pivotal role in the pathogenesis of coronary heart disease, stroke, and other vascular disorders, thereby necessitating effective therapy to achieve the lysis of blood clot (Hastuti, 2022; World Health Organization, 2023)

Thrombolytic therapy aims to dissolve thrombi through the activation of the fibrinolytic system. Currently, common thrombolytic agents include streptokinase, urokinase, and nattokinase. Although effective, the administration of these agents has been reported to induce various adverse effects, such as hemorrhage, allergic reactions, hypotension, and thrombocytopenia, in addition to relatively high therapeutic costs (Shen et al., 2021; kotb et al., 2019). Consequently, there is an imperative need to identify alternative thrombolytic agents that are safer, more cost-effective, and derived from natural sources.

Medicinal plants are known to contain secondary metabolite compounds, such as flavonoids, saponins, and tannins, which possess potential thrombolytic activity. Flavonoids play a role in increasing tissue plasminogen activator while decreasing plasminogen activator inhibitor-1 (PAI-1), thereby supporting the fibrinolysis process (Azzahra et al., 2022). Saponins and tannins have also been reported to contribute to the dissolution of blood clots through interactions with fibrin structures and cell membranes. (Ahsan, 2021; Ghazanfari et al., 2021).

The spider plant (*Chlorophytum* spp.) is an ornamental plant known to contain flavonoids, saponins, and tannins, and it possesses various biological activities. (Isnawati, 2021). Previous studies reported that the root portion of *Chlorophytum* spp. exhibits an *in vitro* thrombolytic activity of $29,4 \pm 3,57\%$ (Riaz et al., 2018). However, scientific studies regarding the thrombolytic activity of spider plant leaf extract remain limited. Therefore, this study was conducted to evaluate the *in vitro* thrombolytic activity of spider plant leaf extract (*Chlorophytum* spp.) as a potential natural-based alternative for thrombolytic agents.

Methodology

Research methods

This study was an experimental laboratory research employing an *in vitro* approach with a post-test only control group design to evaluate the thrombolytic activity of ethanol extract of spider plant leaves (*Chlorophytum* spp.). Fresh spider plant leaves

were collected, washed thoroughly under running water, and air-dried for seven days at room temperature away from direct sunlight. The dried leaves were then ground into powder and sieved to obtain a homogeneous simplicia powder.

The extraction process was carried out using the maceration method. A total of 100 g of powdered simplicia was immersed in 1000 mL of 96% ethanol in a dark glass container and macerated for 3 × 24 hours with occasional stirring. The filtrate obtained was filtered through filter paper and concentrated using a rotary evaporator at 40–50°C to obtain a thick ethanol extract. The percentage yield of the extract was calculated using the formula:

$$\text{Yield (\%)} = (\text{Weight of thick extract} / \text{Weight of dried simplicia}) \times 100$$

Phytochemical screening of the ethanol extract was subsequently performed to identify the presence of secondary metabolites. Flavonoids were tested using 10% NaOH reagent, alkaloids using Dragendorff, Wagner, and Mayer reagents, saponins by the foam test, tannins using 1% FeCl₃, and phenolic compounds using FeCl₃ reagent. The phytochemical screening results were included in the Results and Discussion section.

The thrombolytic activity assay was performed using human venous blood clot samples. Five treatment groups were prepared, consisting of a negative control (distilled water/aquadest), a positive control (nattokinase), and ethanol extract concentrations of 0.5%, 1%, and 2%. Each treatment was conducted in triplicate. Nattokinase was selected as the positive control because of its well-established fibrinolytic activity and was prepared at a concentration of 10 mg/mL.

Microtubes were weighed before use and then filled with 600 µL of venous blood. The blood samples were allowed to clot for 30 minutes at room temperature. After clot formation, serum was carefully removed by centrifugation, and the clot-containing microtubes were weighed to determine the initial clot weight. Subsequently, 100 µL of each treatment solution was added to the corresponding microtube. The samples were incubated at 37°C for 24 hours.

Following incubation, the remaining clot was weighed to determine the final clot weight. Thrombolytic activity was assessed by measuring the reduction in clot mass before and after treatment. The percentage of clot lysis was calculated using the following formula:

$$\text{Clot lysis (\%)} = [(\text{Initial clot weight} - \text{Final clot weight}) / \text{Initial clot weight}] \times 100$$

The primary materials included spider plant leaves (*Chlorophytum* spp.), human venous blood, 96% ethanol, distilled water, nattokinase, NaOH, FeCl₃, Dragendorff reagent, Wagner reagent, Mayer reagent, and hydrochloric acid. The instruments used

consisted of analytical balances, microtubes, centrifuge, incubator, rotary evaporator, blender, laboratory glassware, and filter paper.

Data were analyzed descriptively and presented as mean percentages of clot lysis for each treatment group. The thrombolytic activity of the extract was evaluated by comparing the average clot lysis percentages among the negative control, positive control, and extract-treated group

Results and discussion

Phytochemical screening results indicated that the ethanol extract of spider plant leaves (*Chlorophytum* spp.) contains flavonoid, saponin, and tannin compounds, whereas alkaloids were not detected (Table 1). The presence of these secondary metabolite compounds plays a critical role in the biological activities of medicinal plants, including antithrombotic and thrombolytic activities (Ahsan, 2021). Flavonoids and saponins are known to possess the ability to influence hemostasis and fibrinolysis systems, thereby potentially supporting the process of blood clot dissolution (Azzahra et al., 2022).

Table 1. Representative Observation Data Used for Clot Lysis Calculation.

Treatment	Initial Clot Weight (g)	Final Clot Weight (g)	Clot Lysis (%)
Nattokinase	0,631	0,194	43,7
Destilatted Water	0,333	0,290	4,3

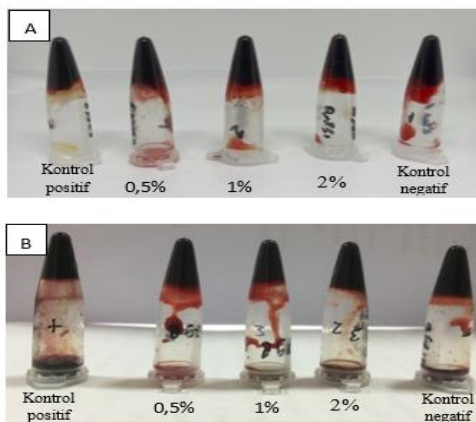


Figure 1. Results of the thrombolytic activity assay (a) before treatment ; (b) after treatment

The thrombolytic activity assay was performed by measuring clot weight before and after treatment. The percentage of clot lysis was calculated using the following formula:

$$\text{Clot lysis (\%)} = [(\text{Initial clot weight} - \text{Final clot weight}) / \text{Initial clot weight}] \times 100$$

Representative observation data demonstrated that the positive control (nattokinase) reduced clot weight from 0.631 g to 0.194 g, resulting in a clot lysis percentage of 43.7%. In contrast, the negative control (distilled water) reduced clot weight from 0.333 g to 0.290 g, corresponding to a clot lysis percentage of 4.3% (Table. 2). These findings confirmed the validity of the thrombolytic assay, as the positive control exhibited substantially greater fibrinolytic activity than the negative control.

Tabel 2. Thrombolytic Activity of *Chlorophytum comosum* Leaf Extract

Replicate	Percentage of clot lysis (%)				
	Control		Extract		
	Nattokinase (+)	Aquadest (-)	0.5%	1%	2%
I	43,7	4,3	2,8	4	5,7
II	43,7	4,3	2,4	3,7	4,8
III	43,7	4,3	3,4	5,1	6,6
Mean	43,7%	4,3%	2,8%	4,2%	5,5%

The concentrations of 0.5%, 1%, and 2% were selected to evaluate the concentration-dependent thrombolytic effect of the extract and were based on previous studies employing graded concentrations of medicinal plant extracts in in vitro thrombolytic assays (Ahsan, 2021). Nattokinase was used as the positive control at a concentration of 10 mg/mL because of its well-established fibrinolytic activity and its ability to directly degrade fibrin, the principal structural component of blood clots.

The thrombolytic activity of *Chlorophytum comosum* leaf extract is presented in Table 1. The ethanol extract exhibited concentration-dependent clot lysis, with mean thrombolytic activities of 2.8%, 4.2%, and 5.5% at concentrations of 0.5%, 1%, and 2%, respectively. The positive control, nattokinase, exhibited the highest thrombolytic activity (43.7%), whereas the negative control (distilled water) showed 4.3% clot lysis. These results indicate that increasing extract concentration enhanced clot dissolution, suggesting a positive dose-response relationship. In vitro thrombolytic activity assays demonstrated that *Chlorophytum comosum* leaf extract is capable of lysing blood clots, with clot lysis increasing proportionally with extract concentration. This concentration-dependent pattern is consistent with previous studies on plant-derived thrombolytic agents, which reported that higher extract concentrations generally produce greater clot lysis activity (Shen et al., 2021).

The thrombolytic activity of *Chlorophytum comosum* leaf extract is likely associated with its flavonoid content. Flavonoids have been reported to enhance tissue plasminogen activator (t-PA) activity and suppress plasminogen activator inhibitor-1 (PAI-1), thereby promoting the conversion of plasminogen to plasmin, the primary enzyme responsible for fibrin degradation (Azzahra et al., 2022). In addition, saponins possess surface-active properties that may alter fibrin structure and facilitate thrombus

breakdown (Ahsan, 2021).

The lower thrombolytic activity of *Chlorophytum comosum* leaf extract compared with nattokinase may be attributed to differences in their mechanisms of action. Nattokinase is a purified proteolytic enzyme that directly hydrolyzes fibrin, whereas the plant extract contains multiple bioactive compounds that act through indirect and more gradual pathways (Hutapea & Rostinawati, 2019). Nevertheless, the observed clot lysis activity supports previous findings reported by Riaz et al. (2018), who demonstrated that *Chlorophytum* spp. possesses *in vitro* thrombolytic potential.

Based on these findings, *Chlorophytum comosum* leaf extract shows potential as a natural thrombolytic agent and may be further developed as a complementary therapeutic option for the prevention of thrombotic disorders. However, additional studies involving higher extract concentrations, isolation of active compounds, and *in vivo* investigations are required to confirm its efficacy and safety profile (Shen et al., 2021).

Conclusion

The ethanolic extract of *Chlorophytum* spp. leaves demonstrated *in vitro* thrombolytic activity, with the percentage of blood clot lysis increasing in a dose-dependent manner. Secondary metabolites, including flavonoids, saponins, and tannins, are hypothesized to contribute to the thrombolytic activity of the extract through mechanisms related to the fibrinolytic system. Although the thrombolytic activity of the extract remains lower than that of the positive control, nattokinase, these findings suggest that *Chlorophytum* spp. leaves have the potential to be developed as a supportive source of natural thrombolytic agents. Further research involving the purification of active compounds and *in vivo* testing is necessary to ensure its efficacy and safety for clinical use.

Declaration of Competing Interest

The authors declare that they have no competing interests, and the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Reference

- Ahsan, S. (2021). Uji aktivitas antiplatelet, antikoagulan, dan trombolitik alkaloid total daun pepaya (*Carica papaya* L.). *Jurnal Biology*, 8(2), 1–20.
- Azzahra, A., Farhani, N., Syahfitri, W., & Pasaribu, S. F. (2022). Potensi kandungan flavonoid dalam kayu batak sebagai antidiabetes. *Jurnal Kesehatan Masyarakat*, 6(2), 14345–14350.

Ferdous, J., Rahman, M., & Hasan, M. (2021). In vitro thrombolytic activity of medicinal plants: A review. *Journal of Applied Pharmaceutical Science*, 11(3), 1–8.

Ghazanfari, T., Hassan, Z. M., & Ebrahimi, M. (2021). Flavonoids and their role in fibrinolytic activity. *Phytotherapy Research*, 35(4), 1234–1245.

Hastuti, N. D. (2022). Uji aktivitas antiplatelet ekstrak etanol rimpang jahe merah (*Zingiber officinale* var. *rubrum*). Universitas Anwar Medika.

Hutapea, E. E., & Rostinawati, T. (2019). Produksi enzim streptokinase dengan teknologi DNA rekombinan. *Farmaka*, 17(2), 351–358.

Isnawati, U. M. (2021). Pemanfaatan indoor plants untuk kualitas udara. *Batara Wisnu Journal*, 1(1), 85–96.

Kotb, E., et al. (2019). Fibrinolytic enzymes and thrombolytic agents. *Biotechnology Reports*, 23, e00304.

Prasad, S., Gupta, S. C., & Tyagi, A. K. (2020). Natural products as thrombolytic agents. *Biochemical Pharmacology*, 177, 113981.

Rahman, M. A., et al. (2021). Role of saponins in clot lysis activity. *Journal of Natural Medicines*, 75(2), 300–310.

Riaz, M., Anjum, F., Talpur, M. M. A., Pirzada, T., Mahmood, Z., & Yousaf, F. (2018). Thrombolytic activity of medicinal plants. *Sylwan*, 162(9), 157–169.

Shen, M., Wang, Y., Hu, F., Lv, L., Chen, K., & Xing, G. (2021). Thrombolytic agents: Nanocarriers in targeted release. *Molecules*, 26(22), 6892.

World Health Organization. (2023). *Cardiovascular diseases (CVDs)*. WHO Press.

Kumar, V., Abbas, A., & Aster, J. (2020). *Robbins basic pathology* (10th ed.). Elsevier.

Choudhury, A., et al. (2019). Evaluation of thrombolytic activity of plant extracts. *International Journal of Pharmacy*, 15(2), 45–52.

Islam, M. T., et al. (2020). Phytochemical and pharmacological properties of medicinal plants. *Journal of Ethnopharmacology*, 249, 112–130.

Das, A., et al. (2021). Herbal thrombolytic agents: Mechanism and applications. *Pharmacognosy Reviews*, 15(29), 45–60.

Khan, I. N., et al. (2019). Evaluation of natural thrombolytic agents. *Bangladesh Journal of Pharmacology*, 14(3), 101–108.

Sarker, S. D., & Nahar, L. (2020). *Natural medicine: The genus Chlorophytum*. Springer.

Patel, K., et al. (2022). Advances in plant-based thrombolytic therapy. *Frontiers in*

Pharmacology, 13, 889456.

Yadav, R., et al. (2021). Medicinal plants with thrombolytic potential. *Journal of Herbal Medicine*, 27, 100–112