

## In Vitro Thrombolytic Activity Test On Ethyl Acetate Fraction of Herb *Phyllanthus niruri* L

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### Abstract

Atherothrombotic, which is an atherosclerotic inflammation with thrombus, is characterized by damage to blood vessels that causes myocardial infarction and ischemic stroke with thrombolytic treatment. The side effects of thrombolytic treatment are the cause of the importance of alternative medicine from natural ingredients. The ethyl acetate fraction in *Phyllanthus niruri* L. is suspected to have thrombolytic activity due to the content of coumarin and flavonoid bioactive compounds based on research that has been done. The purpose of this study was to determine the thrombolytic activity of the ethyl acetate fraction of *Phyllanthus niruri* L. Thrombolytic activity test is done by measuring the percentage of clot lysis. This study used a true experimental method with a Post Test Only Control Group Design model in 6 treatments, positive control, negative control (PBS and 0.5% DMSO) and four samples for both tests, namely 250 g/mL, 500 g/mL, 1000 g/mL, 2000 g/mL. The results of statistical analysis showed that the data were normally distributed (Shapiro Wilk,  $p > 0.05$ ). The result of the largest clot lysis thrombolytic test was 24.43% (2000ppm) smaller than the positive control 46.33%. The results of the thrombolytic ANOVA test showed that there was no significant difference between treatments,  $p < 0.05$ , namely 0.017 and F Count  $>$  F Table, namely  $0.937 > 3.11$ . Based on the results, it was found that the ethyl acetate fraction of the herb *Phyllanthus niruri* L. had a thrombolytic substance with low potency.

**Keywords:** Ethyl Acetate Fraction, Thrombolytic, *Phyllanthus Niruri* L

### Introduction

According to Chan & Weitz, (2019) The hemostasis system is an important system that controls bleeding by keeping blood flowing. Hemostasis has a very important role for survival that serves to secure blood flow so as to optimize the work of organs and cells in physiological conditions and restore bleeding disorders caused by tissue damage (Murray et al., 2003). Based on basic health research data (Riskesdas) in 2018, as many as 15 out of 1000 people, or about 4.7% of individuals in Indonesia suffer from heart disease. The prevalence of heart and blood vessel disease is increasing from year to year (Marisa, 2022). Some cases today are also found in COVID 19 patients often hypoxia that causes vasoconstriction and decreased blood flow and can cause Pulmonary embolism (PE), cytokine storms and hypercoagulation resulting in thrombosis (Sunggoro & Hasan, 2022).

Disorders of atherosclerotic plaque trigger the occurrence of platelet aggregation and activation of coagulation and culminate in the formation of platelet-rich thrombus thus blocking blood flow a process called atherothrombosis. This process is the main cause of myocardial infarction (MI), ischemic stroke, and acute limb ischemia (Smith, 2012). Meniran (*Phyllanthus niruri* L.) is one of the nutritious plants that are often used by Indonesian people. The results of phytochemical filtering of methanol extract of the herb meniran (*Phyllanthus niruri* L.) showed the presence of a group of flavonoid compounds, saponins, tannins, quonones, triterpenoids

(Masriani & Budi, 2017), coumarin and essential oils. Some compounds have a thrombolytic effect in *L. Phyllanthus niruri* L., namely coumarin and its flavonoids.

Methyl brevilincolincarboxylate and Ellagic Acid, which are coumarin derivatives in *Phyllanthus niruri* L. non-*Phyllanthus* leaves, show inhibitory effects on platelet aggregation associated with decreased concentration of  $Ca^{2+}$  in platelets (Iizuka et al, 2007). *Phyllanthus niruri* L also has Quercetin (Guglielmone et al., 2002; Dar & Tabassum, 2012) Rutin which are flavonoid derivatives with potential thrombolytic agents. Therefore, research is needed to be done to be able to find out thrombolytic activity in the ethyl acetate fraction of the herb *Phyllanthus niruri* L. The study was conducted in vitro using the ethyl acetyl acetate fraction of the herb meniran (*Phyllanthus niruri* L.) with various concentrations for thrombolytic testing.

## **Methods**

### ***Research Time***

This research was conducted at the Integrated Laboratory of the Faculty of Health Sciences, Darussalam Mantingan University in 2021. The duration of the study was 4 months, starting from January 2021 - October 2022.

### ***Tools and Materials***

The tools used are maserator, glass funnel, beaker, measuring glass, stirrer rod, vial, injection spuit, centrifuge tube, vortex, analytical balance, rotary evaporator, KLT chamber, micropipet, microscope (Olympus CX23), filter paper, porcelain cup, pH meter, waterbath, stopwatch, tourniquet.

The materials used are Simplisia Meniran (*Phyllanthus niruri* L.), Aquades, Heparin, Nattokinase, Tween 80, Silica Gel

60 F254, Ethyl Acetate, KOH metanolic, Methanol, phosphate buffer saline (PBS), ammonia 10%, Toluen, Format Acid, Acetone, Ethanol 96%, Magnesium,  $FeCl_3$ , concentrated HCl.

### ***Sample Preparation***

Preparation of 1 kg of *Phyllanthus niruri* L. herbal simplisia in maceration using 50% methanol in 3x24 hours. Liquid extract is evaporated using a rotary evaporator at a temperature of 60° C, 150 rpm until the extract is thick. (6) Extracts of herb supplements are partitioned with a fractionation of liquids ratio of 1:1 with Ethyl Acetate: H<sub>2</sub>O. (8) This study used the true experimental method with the Post Test Only Control Group Design model on 6 treatments, positive control, negative control (PBS and 0.5% DMSO) and 4 samples for both tests namely 250 µg/mL, 500 µg/mL, 1000 µg/mL, 2000 µg/mL. Fractions were identified with color reagent and TLC to find out the content of flavonoids and coumarins.. Thrombolytic Test

The 500 µl blood thrombolytic test is incedated at a temperature of 37° C for 45 minutes after the blood clot is formed, the serum is directly removed without damaging the clot. The clot was given a 100 µl sample of 250 µg/mL, 500 µg/mL, 1000 µg/mL and 2000 µg/mL along with positive control (Nattokinase 10,000 µg/mL), negative control (PBS and DMSO 0.5%) and incutated 37° C for 90 minutes. The result is the percentage of lysis before-after.

The research conducted on this human subject received etical approval by the Research Ethics Committee of the Faculty of Medicine, University of Muhammadiyah Surakarta NO.3830/B.2/KEPK-FKUMS/XI/2021. Sampling has been tested with complete information by the subject and will be conducted by experts darussalam Medical Center (DMC) University darussalam Gontor who have been certified.

## **Results and Discussion**

## Rendemen and Phytochemical Tests

The extraction of 50% methanol obtained as much as 44.8 grams with a yield of 8.96%. While the results of fractionation obtained a water fraction yield of 84.59% and a yield of ethyl acetate fraction as much as 10.04%.

Identify active compounds of ethyl acetate and *Phyllanthus niruri* L. water fractions with color silverware and TLC.

Table 1. The results of phytochemical screening of ethyl acetate fraction of the herb *Phyllanthus niruri* L

Phyllanthus niruri L				
	Secondary metabolite compounds		EA	H <sub>2</sub> O
TLC	Coumarin (Ellagic Acid) 0.21(11)	Rf	+	-
	Flavonoid (Quercetine) 0.56(12)	Rf	+	-

Description: (+) : There is a chemical compounds

(-): There is no chemical compounds

Positive results of coumarin compounds and flavonoids in TLC tests and color reagents were in the ethyl acetate fraction. Therefore, ethyl acetate fraction is thought to have stronger thrombolytic compounds compared to water fractions.

## Thrombolytic Test Results

In vitro thrombolytic activity testing is done using blood clot assay. The positive control used is the enzyme nattokinase. Nattokinase was chosen as a positive control because it has similarities Mechanisms with some content in ethyl acetate fractions are flavonoids and coumarins that can degrade fibrin and fibrinogen. Flavonoids work by increasing the formation and release of Plasminogen Activator (t-PA) tissue and lowering levels of PAI-1 (Plasminogen activator Inhibitor-1) (Uddin et al., 2015) While coumarin can break down phospholipidyide which will raise the levels of u-PA (Urokinase Plasminogen Activator) that function in fibrinolysis (Mochizuki et al., 2002).

The ethyl acetate fraction of the herb meniran demonstrated the ability to lysule blood clots with coumarins that can raise uPA and flavonoid levels resulting in inhibition of TNF- $\alpha$ -induced PAI-1 production, and this treatment resulted in a significant reduction of the PAI-1 ratio in TPA inhibition (Kim & Bae, 2012). Measurements of clot lysis are directly proportional to the amount of extract and incubation time.

The ethyl acetate fraction showed that the lowest concentration of 250 ppm had low thrombolytic activity compared to 1000 ppm and 500 ppm and the highest thrombolytic effect was at the first concentration of 2000 ppm.

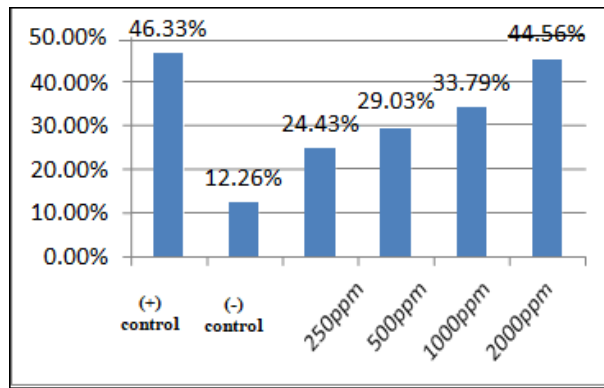


Figure 1. Graph Percentage of Lysis Of Frozen Fraction Ethyl Acetate Herb Phyllanthus niruri L.

One Way Anova test result with a gain of 0.017 with a significance value of  $p < 0.05$  which means a significant effect in the difference between treatments.

Table 2. Results of One Way Anova Analysis of Ethyl Acetate Meniran Fraction Based on Percentage of Lysis of Clots

	Sum of Squares		Mean Square	F	Sig.
Between Groups	2468.195	5	493.639		
Within Groups	1358.020	12	113.168	4.362	.017
Total	3826.215	17			

Based on the F test, the F Table is obtained by 3.11 which can be shined from the table in Appendix 5. The results obtained by F calculated 0.937 ( $F_{\text{Calculate}} < F_{\text{Table}}$ ), and the significance value of  $0.347 > 0.05$  then  $H_0$  was accepted and  $H_1$  was rejected so that it concluded that differences between concentrations simultaneously had no significant effect on the thrombolytic test. The results can be concluded that there is a simultaneous meaningful difference between concentrations with higher concentrations, the higher the thrombolytic activity.

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### Conclusion

The ethyl acetate fraction of the herb Phyllanthus niruri has thrombolytic activity based on the percentage of lysis of clots with the higher the concentration of treatment, the stronger its effectiveness.

### Suggestion

1. Test the thrombolytic activity of the ethyl acetate fraction of the herb Phyllanthus niruri L. in vivo
2. Toxicity tests are performed against the ethyl acetyl acetate fraction of the herb Phyllanthus niruri L. both acute, subchronic and chronic toxicity.

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