



## A Zymography Analysis Of Protease Activity Present In Commercial Fibrinolytic-Lumbrokinase Agents In Indonesia

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### Abstract

**Background:** Fibrinolytic agents are useful in eliminating blood clotting in several disorders. Beside Nattokinase, Lumbrokinase is often used the main ingredient of fibrinolytic agent commercially.

**The objectives** of this study were to inventory and categorise fibrinolytic agents available commercially based on the kind of their bioactive compound (fibrinolytic enzyme), and to do Zymography analysis to compare the proteolytic activity of several Lumbrokinase containing agents.

**Method:** Fibrinolytic agents available commercially were collected from the drug store in Jakarta. They were classified based on the information available in the leaflet or in the drug box. The Casein-Proteolytic Zymograph was carried out for the Lumbrokinase containing agents.

**Result:** The fibrinolytic agents in commercially could be categorised into two types, Lumbrokinase and Nattokinase containing enzymes. Zymography analysis showed that Lumbrokinase-fibrinolytic agents have different strength. Their PAGE profile showed that several bands were missing.

**Conclusion:** Two fibrinolytic enzymes are used for main ingredient, Lumbrokinase and Nattokinase. Fibrinolytic agents with Lumbrokinase are different in their strength and their protein profile is not exactly similar.

**Keywords:** fibrinolytic agent, lumbrokinase, nattokinase, zymography analysis.

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### Introduction

Fibrinolytic enzyme is an enzyme which able to catalyse the breakdown or degradation of fibrin (Fibrinolysis). As insoluble long fibrous chains protein, fibrin is main constituent of the blood clot and is produced in response to bleeding. Fibrin is formed from fibrinogen, a soluble protein that is

produced by the liver and found in blood plasma. When tissue damage results in bleeding, fibrinogen is converted at the wound into fibrin by the action of thrombin, a clotting enzyme. Fibrin molecules then combine to form long fibrin threads that entangle platelets, building up a

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spongy mass that gradually hardens and contracts to form the blood clot (Ahmed et al., 2008).

In contrary cases, fibrinolysis is a physiologic process maintaining patency of the microvasculature. Maladaptive over activation of this essential function (hyperfibrinolysis) is proposed as a pathologic mechanism of trauma-induced coagulopathy. Therefore, the shutdown of fibrinolysis has also been observed as a pathologic phenomenon (Moore et al. 2014).

Fibrin as the main protein component of blood clot can be lysed by fibrinolytic enzymes. Therefore, these enzymes can be used in the treatment of thrombosis. Oral thrombolytic therapy was reported the enhancement of fibrinolytic activity in the plasma (Sumi et al.1990). Two main enzymes have been used so far as medicine, namely Nattokinase and Lumbrokinase. Nattokinase is obtained from Natto. Lumbrokinase is obtained from earthworm (*Lumbricus rubellus*) (Nakayoshi et al., 1993 ). Other plasminogen activator molecules, namely, urokinase, streptokinase, and tissue plasminogen activator, can be used in thrombolytic therapy but are expensive and also have unintended physiological effects like bleeding complication, low fibrin specificity, and short half-lives (Vijayaraghavan et al. 2017).

Fibrinolytic enzymes are used as thrombolytic agents to treat cardiovascular diseases and stroke. Hence, the search for the effective and safe thrombolytic agent from different bio-sources keeps growing worldwide. Study reports are available on fibrinolytic enzymes from sources,

such as Natto and Earthworm. Fibrinolytic enzymes have the ability to inhibit blood coagulation and are able to degrade the fibrin ( Vijayaraghavan et al. 2017)

The objectives of this study were to categorise the fibrinolytic agents available commercially in Indonesia, and to evaluate and to compare the protein profile and proteolytic activity of several agents by using Zymography analysis.

### Materials and methods

**Commercial fibrinolytic based drug:** The fibrinolytic agents sold in the drug store in Jakarta were collected and noted the information available in the brochure/box.

**Casein zymography assay.** For the zymography assay, supernatant of the diluted capsul or tablet was collected and mixed with sample buffer followed by electrophoresis on a 10% SDS-PAGE containing 5 mg/ml of casein. The gel was washed with 2.5% Triton-X 100 solution for 2 h and further incubated in the reaction buffer (50 mmol/l Tris-HCl, 5 mmol/l CaCl<sub>2</sub>, 1 µmol/l ZnCl<sub>2</sub> and 1% Triton X-100) for an additional 18 h at room temperature. The gel was then stained with 0.5% Coomassie blue for 9 h, and subsequently immersed with destaining buffer (30% methanol and 10% acetic acid) for 12 h. Images were photographed and the intensity of each band was digitally quantified.

### Results

Two groups of fibrinolytic agents were identified Lumbrokinase and Nattokinase (Table 1).

**Table 1. List of fibrinolytic agents and their characteristics**

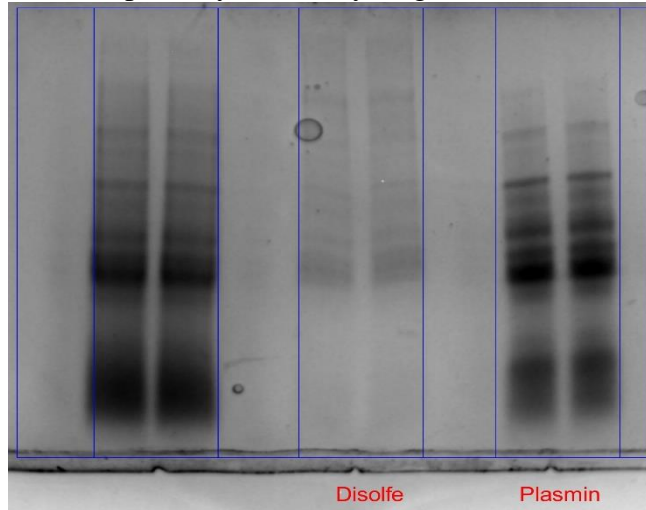
fibrinolytic agent	Available concentration	Dose
Disolf	Lumbricus rubellus 490 mg tablet	1-2 tablet 3 time daily
Plasmin	Lumbricus 250 mg Produce three enzymes as their active compounds, namely Collagenase, fibrinolysin, profibrinolysin activator.	2-3 kapsul 3 time daily
Tromboles	Purified earthworm extract powder 250 mg/capsul	3 kapsul, 1-2 time daily
Trobesco	Nattokinase 22.000 FU/g 10 mg/capsul (setara dengan Nattokinase 1000 FU)	1 kapsul, 1-2 time daily
Fibrinase	Nattokinase (22.000 FU/g) 100 mg	1 kapsul, 1-2 time daily

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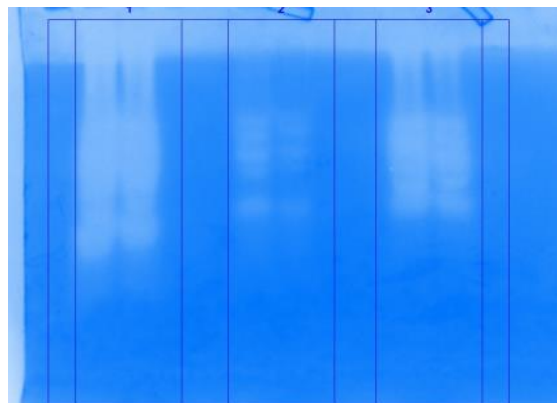
	Bromelain (2.400 GDU/g) 100 mg Papain NF (2.400 USP Unit/g) 100 mg Rutin flavonoid Complex 125 mg White Willowbark Extract 100 mg	
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All are sold as herbal medicine. Five fibrinolytic agents were varied in the kind of enzymes.

The PAGE protein profile of three Lumbrokinase fibrinolytic agents showed different protein concentration. (Figure 1). Not all protein bands have proteolytic activity (Figure 1 and 2).

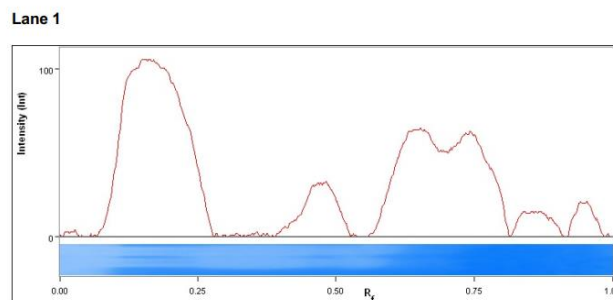


**Figure 1. PAG profile of Tromboles, Disolf and Plasmin**

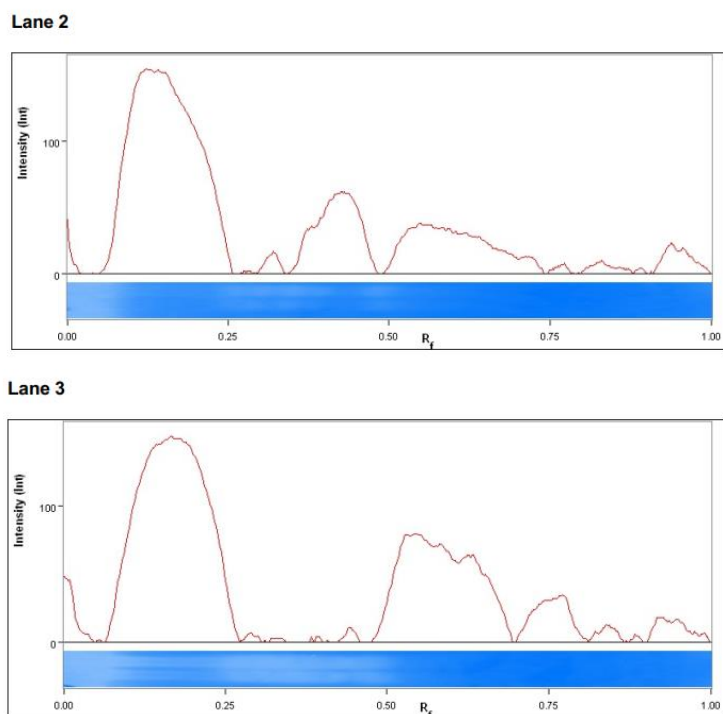


**Figure 2. Zymography**

The largest and lowest did not show proteolytic activity. Scanning of the bands of the Zymograph analysis (Figure 3) showed that there were active similar bands, but several bands were missing or not significantly expressed certain agents.



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**Figure 3. Land and band analysis of the Zymography**

### Discussion

The use of Lumbrokinase and Nattokinase reflex that they are cheaper than other fibrinolytic enzymes and easy to obtain/prepare. Lumbrokinase is obtained from the earthworm (*Lumbricus*). Nattokinase is obtained from Natto. Earthworm and Natto are good and cheap source for the fibrinolytic enzyme.

PAGE analysis and Zymography analysis of the lumbrokinase-fibrinolytic enzymes showed that even extract of earthworm is used, their strength is different. This may be due to the different quality of earthworm extract. These extract contained several bands which are active as proteolytic enzyme. These bands are probably different proteolytic enzyme and may have different functions, such as collagenase, fibrinolysin, profibrinolysin activator

### Conclusion

Lumbrokinase and Nattokinase are the most fibrinolytic enzymes used as the main ingredient of fibrinolytic agents. The quality of Lumbrokinase in different fibrinolytic agents is not similar. Their proteolytic strength and protein profile is not similar.

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