

Evaluation of Thrombolytic Activity of *Zingiber cassumunar* Roxb. and Thai Herbal Prasaplai Formula

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Abstract—The propose of this study was to investigate *in vitro* thrombolytic activity of *Zingiber cassumunar* Roxb. and Prasaplai, a Thai herbal formulation of *Z. cassumunar* Roxb. Herbs were extracted with boiling water and concentrated by lyophilization. To observe their thrombolytic potential, an *in vitro* clot lysis method was applied where streptokinase and sterile distilled water were used as positive and negative controls, respectively. Crude aqueous extracts from *Z. cassumunar* Roxb. and Prasaplai formula showed significant thrombolytic activity by clot lysis of 17.90% and 25.21%, respectively, compared to the negative control water (5.16%) while the standard streptokinase revealed 64.78% clot lysis. These findings suggest that *Z. cassumunar* Roxb. exhibits moderate thrombolytic activity and could play an important role in the thrombolytic properties of Prasaplai formula. However, further study should be done to observe *in vivo* clot dissolving potential and to isolate active component(s) of these extracts.

Keywords—Aqueous extract, prasaplai formula, thrombolytic activity, *Zingiber cassumunar* Roxb.

I. INTRODUCTION

BLOOD clot formation, thrombosis, has been a severe problem of the blood circulation system. Thrombus or embolus obstructs the blood flow by blocking the blood vessel therefore depriving blood and oxygen supply to tissue, leading to tissue necrosis. Atherothrombotic diseases such as acute myocardial or cerebral infarction and stroke are serious consequences of the thrombus formed in blood vessels. Usually thrombolytic agents such as streptokinase (SK), urokinase (UK), or tissue-plasminogen activator (t-PA) [1]-[3], are used to dissolve the formed clots in the vessels, however, these drugs have certain limitations which cause severe and sometime fatal disorders including systemic fibrinolysis, anaphylactic reaction, and bleeding tendency [4]-[6].

Traditional herbs have been used since ancient times to treat many diseases. Herbs are often known as safe because they are natural products. Previous studies have shown that many herbs possessed antithrombotic activity [7]-[11]. For instance,

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methanolic extract of *Umbilicaria esculenta* exhibited both antithrombotic activity *in vivo* and *in vitro* [7]. However, herbs that could be used for thrombolysis has been few reported.

Ayurvedic Prasaplai is a Thai traditional herbal drug for pain treatment. Prasaplai is derived from “Prasa” and “Plai” in which Prasa means 50% in amounts and Plai is a Thai name of *Zingiber cassumunar* Roxb. Prasaplai formula contains 50% *Z. cassumunar* Roxb., while the remaining ingredients consist of equal amounts of each plant including the root of Calamus (*Acorus calamus* L.), the peel of Kaffir lime (*Citrus hystrix* DC.), the bulb of Waanhomdaeng (*Eleutherine americana* (Abul.) Merr.), the bulb of Garlic (*Allium sativum* L.), the fruit of Long pepper (*Piper retrofractum* Vahl.), the fruit of Black pepper (*Piper nigrum* L.), the rhizome of Zedoary (*Curcuma zedoaria* Roscoe.), the rhizome of Ginger (*Zingiber officinale* Roxb.), the seed of Black cumin (*Nigella sativa* L.), and two chemical compounds, sodium chloride and camphor [12]. Usually, it was used for pain relief during menstruation dysmenorrhea [13]. This traditional Thai drug has been described by alternative medicine for treatment of primary dysmenorrhea [14].

Z. cassumunar Roxb, a species of plant in the Zingiberaceae family, is a major ingredient in Prasaplai formula. It is cultivated throughout Southeast Asia [15] and widely used as a Thai traditional herb for treatment of inflammation [16], muscular and joint pain [17], rheumatoid arthritis [18], skin diseases [19], asthmatic symptoms [20], [21], abscesses, wound healing, and menstrual disorders, as well as in food in Thailand [22]. The chemical compositions of the rhizome of *Z. cassumunar* Roxb. have been studied and revealed that there are phenylbutenoids [23], [24], cassumunaquinones [25], β -sesquiphellandrene [26], cassumunarins [27], [28], β -sitosterol, terpinen-4-ol [29], [30], triquinacene 1,4-bis(methoxy), (E)-1-(3,4-dimethoxyphenyl)buta-1,3-diene, (E)-1-(3,4-dimethoxyphenyl) but-1-ene, and (Z)-ocimene [19]. To date, no studies have investigated the thrombolytic properties of *Z. cassumunar* Roxb. and its formula, Prasaplai. With the aim to evaluate thrombolytic activity of the aqueous extracts from *Z. cassumunar* Roxb. and Prasaplai formula, we investigated the thrombolytic activity by using *in vitro* model.

II. MATERIALS AND METHODS

A. Preparation and Extraction of Plant Material

The rhizome of *Z. cassumunar* Roxb. and the 9 raw herbs composing the Prasaplai formula were purchased from a

traditional herb market at Pathum Thani province, Thailand. The plants were cleaned, cut into small pieces, air-dried, and then ground to powder. Prasaplai formula was prepared from 12 components including 50% of the rhizomes powder of *Z. cassumunar Roxb.* (81 parts), each eight parts of *C. hystrix* DC. (peel), *A. calamus* L. (root), *A. sativum* L. (bulb), *E. americana* Merr. (bulb), *P. nigrum* L. (fruit), *P. retrofractum* Vahl. (fruit), *Z. officinale* Roxb. (rhizome), and *C. zedoaria* Roscoe. (rhizome), *N. sativa* L. (seed), and 2 chemical compounds which are sodium chloride (8 parts), and camphor (1 part) [12], [31]. The extraction was performed using boiling distilled water decoction technique. The powder portion of *Z. cassumunar* Roxb. or Prasaplai formula (10% w/v) were soaked in boiling distilled water for 30 minutes at room temperature with occasional stirring. The solution was filtrated through Whatman's filter paper and the filtrate thus obtained was concentrated by lyophilization [32]. The crude extracts of *Z. cassumunar* Roxb. and Prasaplai formula were stored at -20°C until used. The percentage of yield extraction and physical appearance of the crude extracts of *Z. cassumunar* Roxb. and Prasaplai formula were shown in Table I. 5 mg crude extract was suspended in 1 ml distilled water to get a solution of 5 mg/ml.

TABLE I

THE PHYSICAL APPEARANCES AND QUANTITY OF *Z. CASSUMUNAR* ROXB. AND PRASAPLAI FORMULA EXTRACTS OBTAINED AFTER EXTRACTION

Crude extracts	Physical appearance	Yields (%w/w)
<i>Z. cassumunar</i> Roxb.	Dark yellow powder	7.01
Prasaplai formula	Brown powder	3.92

B. Streptokinase (SK)

The commercially available lyophilized vial (Streptase®, CSL Behring GmbH, Marburg, Germany) of 1,500,000 I.U., 5 ml sterile distilled water was added. SK suspension 100 µl (30,000 IU) was used as a standard for *in vitro* clot lysis method as described by Prasad et al. [33].

C. Blood Sample

The venous blood samples were collected from 10 healthy human volunteers (five male, five female, aged 20-35 years) by maintaining aseptic condition without a history of oral contraceptive or anticoagulant therapy. The study design and informed consent form for the volunteers were approved by the Committee on Human Rights Related to Human the Experimentation of Western University, Kanchanaburi 70170, Thailand (reference number WTU2557-00172).

D. Thrombolytic Activity

To determine the thrombolytic activity, 6 ml of peripheral venous blood were collected from each healthy volunteer. Twelve 0.5 ml aliquots of whole blood were transferred to pre-weighed 1.5-ml microcentrifuge tubes. The clot formation was enhanced by incubation of the blood-containing tube at 37°C for 45 minutes. After complete incubation, serum was removed from the blood clot using a Pasteur pipette. The weight of blood clot was determined by subtracting weight of clot containing tube from the empty tube. A total of 100 µl of

SK as a positive control, and 100 µl of sterile distilled water as a negative control, along with 100 µl of 5 mg/ml aqueous extracts of *Z. cassumunar* Roxb. and Prasaplai formula were separately added to the microcentrifuge tubes. The clot lysis of the sample was evaluated after incubation at 37°C for 90 minutes. After incubation, the solubilized clot was separated and the tubes were again weighed to observe the remained clot. The thrombolytic activity was expressed as percentage of clot lysis [33], [34].

$$\% \text{ clot lysis} = \frac{\text{Weight of clot} - \text{Weight of remained clot}}{\text{Weight of clot}} \times 100 \quad (1)$$

E. Statistical Analysis

Data obtained were analyzed using GraphPad Prism 5 version 5.01 (GraphPad Software Inc. La Jolla, CA, USA). All values are expressed as mean±standard error of the mean for three replicates. Data were analyzed by one-way ANOVA and the statistical significance differences were analyzed using the paired t-test. $p < 0.05$ was considered statistically significant.

TABLE II

EFFECT OF *Z. CASSUMUNAR* ROXB., PRASAPLAI FORMULA, SK, AND DISTILLED WATER ON DISSOCIATION OF CLOTS PREPARED FROM BLOOD OF NORMAL INDIVIDUALS

Sample (n=10)	% Clot Lysis			
	DW (Negative control)	SK (Positive control)	ZC (5 mg/ml)	PSL (5 mg/ml)
1	3.54	54.88	14.64	21.69
2	5.23	65.95	17.02	21.80
3	4.62	78.54	17.82	21.98
4	3.84	64.32	10.94	20.11
5	2.90	53.79	13.09	20.63
6	4.33	66.17	20.42	25.79
7	8.12	83.50	29.64	38.22
8	5.80	62.57	17.59	24.78
9	3.36	58.76	11.75	21.91
10	9.89	59.35	26.07	35.15
Mean±SEM	5.16±0.71	64.78±3.04	17.90±1.92	25.21±2.00

DW=sterile distilled water, PSL=Prasaplai formula, ZC=*Zingiber cassumunar* Roxb.

III. RESULTS AND DISCUSSION

Additional of 100 µl SK, a positive control (30,000 I.U.), to the clot showed 64.78±3.04% clot lysis, whereas sterile distilled water (negative control) showed only negligible clot lysis (5.16±0.71) in Table II. The mean difference of clot lysis percentage between positive and negative control was significant ($p < 0.0001$). The thrombolytic comparison of positive control with negative control indicated that the blood clot did not dissolve when distilled water was added to the clot. *In vitro* thrombolytic activity of crude aqueous extracts from *Z. cassumunar* Roxb. and Prasaplai formula were determined. The extracts from *Z. cassumunar* Roxb. and Prasaplai formula showed 17.90±1.92% and 25.21±2.00% of clot lysis, respectively. The mean difference between negative control and *Z. cassumunar* Roxb. was significant ($p < 0.05$), while Prasaplai formula was statistically more significant ($p < 0.0001$) in Table III and Fig. 1.

This study indicated that both aqueous extracts from *Z.*

cassumunar Roxb. and Prasaplai formula exhibited moderate thrombolytic activity. Because of Prasaplai formula consists of 50% of *Z. cassumunar* Roxb. as ingredients, the thrombolytic activity of Prasaplai formula could be the thrombolytic properties of *Z. cassumunar* Roxb. However, the thrombolytic activity of the extract from Prasaplai formula was approximately 1.41 fold-higher than that of *Z. cassumunar* Roxb. This suggested that active compound(s) of other herbs in Prasaplai formula might be involved in the thrombolytic activity of the extract.

TABLE III
THROMBOLYTIC ACTIVITY (IN TERM OF % CLOT LYSIS) OF *Z. CASSUMUNAR* ROXB., PRASAPLAI FORMULA, SK, AND DISTILLED WATER

Extracts	Mean±SEM (% clot lysis)	p-value when compared to negative control (water)
Distilled water	5.16±2.24	
<i>Z. cassumunar</i> Roxb.	17.90±1.92	<0.05
Prasaplai formula	25.21±2.00	<0.0001
SK	64.78±3.04	<0.0001

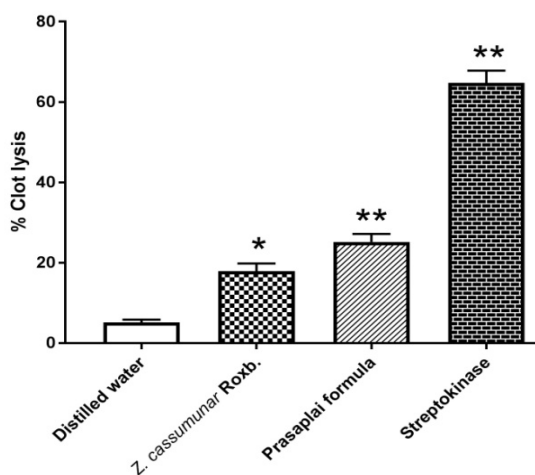


Fig. 1 Comparative thrombolytic activity of *Z. Cassumunar* Roxb Extract Prasaplai formula extract, SK, and distilled water, Prasaplai formula, SK (positive control) and water (negative control) Significant at * $p < 0.05$, and ** $p < 0.0001$ comparing with distilled water

Earlier study revealed that ethanolic leaf extract from *A. calamus* L. showed 13.69% *in vitro* clot lysis. Moreover, there are evidence that the extracts from *A. sativum* L. [35] and *Z. officinale* Roxb. [36] were related to an increased fibrinolytic activity *in vivo* [37]. These herbal ingredients of Prasaplai formula could possibly enhance the thrombolytic activity of the extract. The clot lytic activity of *Z. cassumunar* Roxb. and Prasaplai formula might be a result of one or more active compound(s). By the above obtained results, they can be suggested that *Z. cassumunar* Roxb. and Prasaplai may be useful therapeutic candidates for the prevention or treatment of thrombotic diseases.

IV. CONCLUSION

We have described the *in vitro* thrombolytic activity of

crude aqueous extracts from *Z. cassumunar* Roxb. and Prasaplai formula, which are beneficial in Thai traditional medicine. This study may have important implications in the treatment of thrombotic diseases. Furthermore, this finding may indicate the possibility of developing novel thrombolytic compounds from *Z. cassumunar* Roxb. and Prasaplai formula. Further studies are ongoing to isolate their bioactive compounds responsible for thrombolytic activity and a dose-response relationship study *in vivo* model.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with this study.

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