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Fibrinolytic Activity of Thai Indigenous Vegetables

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ABSTRACT

Thai indigenous vegetables having high antioxidant activity were selected and their fibrinolytic activities were screened. Among them, *Careya sphaerica* Roxb., *Anacadrium occidentale* Linn., and *Barringtonea acutangula* Linn. showed very high fibrinolytic activity followed by *Bonea macrophylla* Griff., *Azadirachta indica* Juss. var. siamensis, *Gymnema inodorum* Pecne. and *Tricholoma crassum* Berk. The activity was significantly affected by heating; all the samples lost their fibrinolytic activities upon heating at 100°C for 10 min. *Tricholoma crassum* Berk., in particular, showed relatively high stability upon heating. Upon 5 min heating, 43% of its activity remained stable. Comparing the activity ratio between fibrinolysis and proteolysis (F/P ratio) of the vegetable extracts, *Tricholoma crassum* Berk. and *Careya sphaerica* Roxb. showed very high F/P ratio indicating their extracts acted as fibrinolytic enzymes.

Key words: Thai indigenous vegetable, fibrinolytic activity, proteolytic activity, thermal stability

INTRODUCTION

The number of patient suffering from cardio vascular disease (CVD) is increasing every year worldwide. This phenomenon is caused by inadequate diet. Fast foods of American style and processed foods have made many people to give up their healthy traditional diet habit. We noted that post-operative venous thrombosis is rare and the incidence of thromboembolism is also low among Thais (Diagosnostic lists, 1970). Unfortunately, even in Thailand the number of heart disease patients showed drastic increase from 101.7 / 100,000 population in 1993 to 285.4/ 100,000 population in 2000 (WHO, 2003). Considering the number only counted the ones who visited hospital, 2 to 3 times of the number safely presumed to have blood circulation problems.

One of the major causes of blood circulation problem is the formation of blood clots. Blood clots (fibrin) are formed from fibrinogen by thrombin (EC 3.4.21.5) and are lysed by plasmin (EC 3.4.21.7), which is activated from plasminogen by tissue plasminogen activator (tPA). Although fibrin clot formation and fibrinolysis are maintained in balance by the biological system, thromboses, such as myocardial infarction, occur when clots are not lysed as a result of a disorder of the balance (Voet and Voet, 1990).

To treat thrombosis patient, intravenous administration of urokinase and streptokinase has been widely used for thrombosis therapy. However, this treatment has disadventage because the enzymes have a low specificity to fibrin and are expensive. tPA has been developed to treat

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thrombosis because of its efficacy and stronger affinity to fibrin (Sherry, 1987).

Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase was one example, which has been reported to enhance fibrinolytic activity in plasma and the production of tPA (Sumi *et al.*, 1990). Nattokinase was found to be the same as subtilisin NAT which is produced from Bacillus NAT in the traditional Japanese fermented food, natto (Nakmura *et al.*, 1992).

Few dietary products have demonstrated the property to enhance fibrinolytic activity such as pineapple (Taussig and Batkin, 1988), ginger (Verma and Bordia, 2001), onion (Nagda *et al.*, 1983), garlic (Nagda *et al.*, 1983; Garcia-Gomez and Sanchez-Muniz, 2000), capsicum (Visudhiphan *et al.*, 1982) and mushroom (Choi *et al.*, 1999). If every day diet is composed of such food materials and proper amounts are ingested, it would diurnally activate fibrinolysis. As a result it would cause high fibrinolytic activity and thus prevent thromboembolism.

In this study we selected Thai indigenous vegetables of high antioxidant activity based upon the previous study (Trakoontivakorn and Saksitpitak, 2000; Rukariyatham and Chenwithisuk, 2002). Most of these vegetables have been consumed commonly among Thai people. We screened these vegetables for fibrinolytic activity and characterized the enzymes to figure out its action *in vivo*.

MATERIALS AND METHODS

Materials

Thai indigenous vegetables used for this study was listed in Table 1. All the samples were collected in the form of fresh product and packed in plastic bag. The samples were stored and carried in ice chest to the laboratory. Samples were kept frozen until use.

Preparation of sample

Frozen vegetable of 50g was homogenized with methanol (150ml) followed by filtration. Residual was homogenized and filtered as above. Filtrates were pooled and adjusted to 300ml with methanol. Concentration was carried out at 45°C with rotary vacuum evaporator to three fold. The concentrate was stored in a -20°C freezer until fibrinolytic activity assay.

Fibrinolytic activity assay

Astrup method was modified for screening purpose (Astrup and Mullertz, 1952). Fibrinogen (Sigma F-8630 from bovine plasma) was dissolved with 50mM sodium borate buffer (pH 7.9) to give a final concentration of 0.006g/ml. The suspension was kept at 37°C to make it soluble. Aliquots of 10ml were placed onto petridish and 50µl of thrombin (200 unit/ml) was added, followed by swift mixing. The plate was solidified for 30min. Holes (2mm diameter) were punched with capillary tube using vacuum suction and sample solution (3µl) was added into the hole followed by incubation at 37°C. Methanol was used as a control. After 18 h incubation the diameter of clear zone was measured. For screening purpose, fibrinolytic activity (FA) was expressed as the area (mm^2) of clear zone.

RESULTS AND DISCUSSION

Screening of fibrinolytic activity

Seventeen vegetables of high antioxidant activity were evaluated for fibrinolytic activity. Most of vegetables tested showed significant fibrinolytic activity (Table 2). Among them, seven vegetables showed strong firbrinolytic activity: *Careya sphaerica* Roxb., *Anacadrium occidentale* Linn., *Barringtonea acutangula* Linn., *Bonea macrophylla* Griff., *Azadirachta indica* Juss. var. siamensis, *Gymnema inodorum* Pecne. and *Tricholoma crassum* Berk. This result could explain the low incidence of CVD cases among Thai

Table 1 List of Thai indigenous vegetables used for the screening of fibrinolytic activity.

Scientific name	Common name	Local name	Place of collection	Time of collection
Anacadrium occidentale Linn.	Cashew	Mamung-himmaparn	Maha Sarakham	June
Antidesma acidum Retz.		Ma mao	Lampang	June
Azadirachta indica Juss. Var.siamensis	Neem tree	Sa dao	Lampang	June
Barringtonea acutangula Linn.	Indian oak	Jik/ Kra don nam	Khon Kaen	June
Bonea macrophylla Griff.	Plum mango	Yod maprang	Chanthaburi	July
Caesalpinia mimosoides Lamk.		Nham pu ya	Lampang	June
Careya sphaerica Roxb.		Kra don bok	Kalasin	June
Cratoxylum formosum Dyer.		Phak tew	Lampang	June
Gymnema inodorum Pecne.		Chiang da	Lampang	June
Jussiaea repens Linn.	Creeping water primrose	Pang puay nam	Maha Sarakham	June
Morus alba Linn.	Mullberry	Mohn	Lampang	June
Ocimum gratissium Linn.		Kra prao chang	Bangkok	June
Parkia speciosa Hassk.		Sa tor	Bangkok	June
Passiflora foetida Linn.		Tam leung tong	Maha Sarakham	June
Piper sarmentosum Roxb.	Variegatum	Cha plu	Lampang	June
Tricholoma crassum Berk.		Hed chan/ Hed teen rad	Maha Sarakham	June
Zingiber montanum Roxb.	Cassumunar ginger	Plai	Lampang	June

people to some extent. Examples of fibrin plate were shown in Figure 1.

Effect of temperature on the fibrinolytic activity

The extracts of 7 vegetables were selected for further study: *Careya sphaerica* Roxb., *Anacadrium occidentale* Linn., *Barringtonea acutangula* Linn., *Bonea macrophylla* Griff., *Azadirachta indica* Juss. var. siamensis, *Gymnema inodorum* Pecne. and *Tricholoma crassum* Berk. Each extract was placed at 100°C in water bath for 1, 5 or 10 min, followed by rapid cooling in ice water. Relative fibrinolytic activity (RFA) was expressed as follows:

$$\text{RFA (\%)} = \frac{\text{Area of clears zone after heat treatment}}{\text{Area of clears zone before heat treatment}} \times 100$$

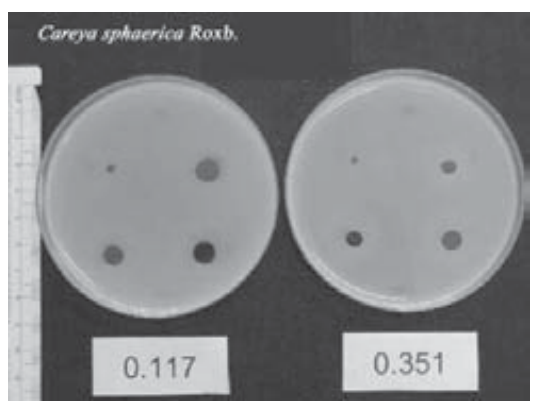

Figure 1 Fibrin plate showing fibrinolytic activity of *Careya sphaerica* Roxb.

(Number below the plates represent NaCl concentrations of buffer; for both plates, the upper left holes were control, the others were extracts of *Careya sphaerica* Roxb.)

Table 2 Screening of Thai indigenous vegetables for fibrinolytic activity.

Scientific name	Local name	FBA (mm^2)
Anacadrium occidentale Linn.	Mamung-himmaparn	38.5
Antidesma acidum Retz.	Ma mao	12.6
Azadirachta indica Juss. var. siamensis	Sa dao	22.1
Barringtonea acutangula Linn.	Jik/ Kra don nam	30.2
Bonea macrophylla Griff.	Yod maprang	19.6
Caesalpinia mimosoides Lamk.	Nham pu ya	9.6
Careya sphaerica Roxb.	Kra don bok	50.2
Cratoxylum formosum Dyer.	Phak tew	9.1
Gymnema inodorum Pecne.	Chiang da	22.1
Jussiaea repens Linn.	Pang puay nam	3.8
Morus alba Linn.	Mohn	4.2
Ocimum gratissium Linn.	Kra prao chang	9.1
Parkia speciosa Hassk.	Sa tor	1.5
Passiflora foetida Linn.	Tam leung tong	11.9
Piper sarmentosum Roxb.	Cha plu	7.5
Tricholoma crassum Berk.	Hed chan/Hed teen rad	17.3
Zingiber montanum Roxb.Young rhizome	Plai	9.1
Zingiber montanum Roxb.Mature rhizome	Plai	12.6

Table 3 Effect of temperature on the fibrinolytic activity of Thai vegetables.

Name	Relative fibrinolytic activity(%)		
	1min	5min	10min
Careya sphaerica Roxb.	68	23	0
Anacadrium occidentale Linn.	45	21	0
Barringtonea acutangula Linn.	52	8	0
Bonea macrophylla Griff.	60	25	0
Azadirachta indica Juss. var. siamensis	41	10	0
Gymnema inodorum Pecne.	35	15	0
Tricholoma crassum Berk.	72	43	0

All the samples lost their fibrinolytic activity upon heating at 100°C for 10min (Table 3). This means that vegetables with high fibrinolytic activity should not be prolong heated. *Tricholoma crassum* Berk., in particular, showed relatively high stability upon heating as compared with the other tested vegetables. Upon 5 min heating, 43% of its activity was remained. Regarding culinary application, *Careya sphaerica* Roxb., *Anacadrium occidentale* Linn., *Barringtonea acutangula* Linn., *Bonea macrophylla* Griff. and *Azadirachta indica* Juss. var. siamensis are traditionally served in the form of raw accompaniment to chili dips or curry. The others, *Gymnema inodorum* Pecne. and *Tricholoma*

Table 4 Comparison of enzyme characteristics with skim milk and fibrin plate.

Name	Fibrinolysis (mm^2)	Proteolysis (mm^2)	F/P
Careya sphaerica Roxb.	50.2	8.0	6.23
Anacadrium occidentale Linn.	38.5	84.9	0.45
Barringtonea acutangula Linn	30.2	60.1	0.50
Bonea macrophylla Griff.	27.3	10.2	2.67
Azadirachta indica Juss. var. siamensis	22.1	11.3	1.96
Gymnema inodorum Pecne.	22.1	44.2	0.50
Tricholoma crassum Berk.	19.6	1.5	13.08

crassum Berk., are ingredients in Northern or North-eastern curries. To obtain the maximum potency, these vegetables should be consumed raw. If blanching process would be employed for hygienic purpose, heating time should not exceed 1 min.

Characteristics of fibrinolytic enzymes from selected vegetables

Sometimes plant proteases cause solublilization on a fibrin plate. Agar plate containing skim milk (1.0%) was used to determine proteolytic activity of vegetable extracts. Clear zone of skim milk plate was compared with fibrin plate (Table 4). *Tricholoma crassum* Berk. and *Careya sphaerica* Roxb. showed very high F/P ratio indicating the extracts from them acted as fibrinolytic enzymes. F/P ratio below 1.0 indicated proteolysis occurrence rather than fibrinolysis as in the case of *Anacadrium occidentale* Linn., *Barringtonea acutangula* Linn and *Gymnema inodorum* Pecne. Further research is in progress to elucidate the characteristics of fibrinolytic enzymes from *Tricholoma crassum* Berk. and *Careya sphaerica* Roxb.

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