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Screening for Antioxidant Activity in Eighteen Local Northeastern Vegetables using Silica Gel Thin-layer Chromatography Followed by Spraying with DPPH

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Abstract

Antioxidant has been used as protection of autoxidation. In order to search for antioxidant activity, 18 types of vegetable from Northeastern Thailand, i.e. *Strychnos minor* Dennst., *Asparagus racemosus* willd., *Blumea napifolia* DC., *Stephania erecta* Linn., *Momordica sp*., *Aganosma marginata* G. Don, *Cassia tora* Linn., *Pluchea indica* Less, *Ottelia alismoides* (L.) Pers., *Blumea sp*., *Solanum nigrum* Linn, *Phyllanthus amarus* Schumach & Thonn., *Emilia sonchifolia* (Linn.) DC., *Droogmansia godefroyana* (Kuntze) Schindl, *Scoparia dulcis* Linn., *Kaempferia galanga* Linn., *Monordica cochinchinensis* (Lour) Spreng., and *Monihoty esculenta Crantz*, were collected. The methanolic extracts were screened for antioxidant activity using silica gel thin-layer chromatography followed by spraying with 2,2–diphenyl-1-picrylhydrazyl hydrate (DPPH). The results showed that 17 types of vegetable from Northeastern Thailand exhibited positive antioxidant but only methanolic extracts of *Ottelia alismoides* (L.) Pers. showed negative antioxidant activity. Moreover, antioxidant components of methanolic extracts of 17 types of vegetable from Northeastern Thailand have the different R_f values with mobile phase acetone, acetone: methanol, and acetone: methanol with the ratio of 1:0, 4:1, and 1:1, respectively.

Keywords: antioxidant, autoxidation, vegetables from Northeastern Thailand

INTRODUCTION

Antioxidants have been used as important protective agents for human health. The crude extracts of various parts of plants have antioxidants and also inhibited anti-inflammatory properties such as *Emilia sonchifolia* leaf extracts (Muko and Ohiri, 2000) and antiviral such as *Phyllanthus* species (Unander *et.al*, 1990). Generally, the antioxidants contained in food are phenolic compounds (phenolic acid and flavonoids), vitamin A, vitamin C, vitamin E, beta-carotene, biofavanoid, zinc, Co-Q A (Block *et al*., 1992; Gillman *et al*.,1995; Vinson, *et al*., 1998) which are nutrient for good condition. Vegetables from Northeastern Thailand are normal diet in common foods. So, in this study, the antioxidant activity of 18 types of vegetable from Northeastern Thailand has been investigated and reported. However, some vegetables have been reported antioxidant activity, for example; *Scoparia dulcis*, used in traditional medicine as an analgesic and antipyretic. The water extract showed *in vitro* antioxidant activity 26.92$\pm$0.43, 34.95$\pm$0.22, and

48.26±0.22% with the concentration of 0.01, 0.1, and 1.0 mg/mL, respectively (Ratnasooriya *et al*., 2005).

In this paper, we also showed the TLC analysis of antioxidant components of the local vegetables from Northeastern Thailand normally used in food.

MATERIALS AND METHODS

Chemicals- All analytical grade organic solvents such as hexane, dichloromethane, chloroform, and methanol were purchased from Labscan Asia (Bangkok, Thailand). Silica gel 60 F 254 for TLC, 20x20 centimeters silica gel was bought from Merck, Germany.

The chemicals for antioxidant activity i.e. 2,2-diphenyl-1-picrylhydrazyl (DPPH), free radical, 95% were purchased from Sigma, Germany.

Plant Materials- The vegetables, included *Strychnos minor* Dennst., *Asparagus racemosus* willd., *Blumea napifolia* DC., *Stephania erecta* Linn., *Momordica sp*., *Aganosma marginata* G. Don, *Cassia tora* Linn., *Pluchea indica* Less., *Ottelia alismoides* (L.) Pers., *Blumea sp*., *Solanum nigrum* Linn., *Phyllanthus amarus* Schumach & Thonn., *Emilia sonchifolia* (Linn.) DC., *Droogmansia godefroyana* (Kuntze) Schindl, *Scoparia dulcis* Linn., *Kaempferia galang* Linn., *Monordica cochinchinensis* (Lour) Spreng., and *Monihoty esculenta* Crantz, were purchased at local markets (Khon Kaen Province, Thailand) and stored at 20^{o} C. All the 18 types of vegetables were authenticated from Northeastern Thailand.

Preparation of Extract - The vegetables were cut and dried at room temperature for 3 days. Each vegetable (200 g) was successively treated with methanol and extracted two times at room temperatures over a period of 78 hours. The extracts were dried under reduced pressure using a rotary evaporator at 50 ^{o} C. The concentrated extracts were stored at 10 ^{o} C.

The extracts were diluted with methanol and used for Thin-layer chromatography (TLC) analysis.

Detection of Antioxidant activity on TLC plate- The samples were applied on a 2.5 mm silica gel 60 F254 TLC plate. After the plate had been developed, it was dried at room temperature and then sprayed with 0.2 mM DPPH in methanol. The DPPH solution was freshly prepared and stored in darkness. After the plate was dried at room temperature, an active spot indicating antioxidant was a colorless spot on the purple background.

RESULTS AND DISCUSSION

Thin layer chromatography analysis of antioxidant activity is very quick and simple method. Moreover, this technique was used as the guide for active component. The methanolic extracts of 17 types of vegetable from Northeastern

Thailand showed antioxidant activity in DPPH and more than one R_f values, but only *Ottelia alismoides* (L.) Pers was not found as shown in Table 1.

Table 1 R_f values of 18 types of vegetable from Northeastern Thailand in three mobile phase solvent systems; acetone: MeOH= 1: 1, acetone: MeOH= 4: 1, and acetone 100%

No.	Vegetable Sample	R_f					
		Acetone: MeOH = 1: 1		Acetone: MeOH = 4: 1		Acetone 100%	
		254 nm	366 nm	254 nm	366 nm	254 nm	366 nm
1	*Solanum nigrum* Linn.	0.05*	0.05*	0.07*	N.D	0.04*	N.D.
		0.13*	0.21*	N.D	0.20*	N.D.	N.D.
		0.83*	0.83*	0.93	N.D	N.D.	N.D.
2	*Phyllanthus amarus* Schumach.& Thonn	N.D.	N.D.	0.07*	0.07*	0.11*	N.D.
		0.85*	0.85*	N.D	0.20*	N.D.	N.D.
3	*Blumea napifolia* DC	0.06*	0.06*	N.D.	N.D.	0.03*	N.D.
		0.15*	0.15*	0.20*	0.20*	N.D.	N.D.
		N.D.	0.82	N.D.	N.D.	N.D.	N.D.
4	*Emilia sonchifolia* (Linn.) DC	N.D.	0.09*	Tail*		0.01*	N.D.
		N.D.	0.71			N.D.	N.D.
		N.D.	N.D.			0.91*	0.91*
5	*Stephania erecta* Linn.	N.D.	0.06*	Tail*		N.D.	N.D.
		N.D.	N.D.			0.11*	N.D.
		N.D.	N.D.			N.D.	N.D.
6	*Scoparia dulcis* Linn.	0.05*	0.05*	Tail*		0.08*	N.D.
		N.D.	0.41*			N.D.	N.D.
7	*Monordica cochinchinensis* (Lour) Spreng	N.D.	0.11*	N.D.		N.D.	0.03*
		N.D.	N.D.	N.D.		N.D.	0.10*
		N.D.	N.D.	N.D.		N.D.	N.D.
8	*Ottelia alismoides* (L.) Pers	N.D.	0.04	N.D.		N.D.	N.D.
		N.D.	0.11	N.D.		N.D.	N.D.
		N.D.	0.56	N.D.		N.D.	N.D.
		N.D.	0.66	N.D.		N.D.	N.D.
		N.D.	0.78	N.D.		N.D.	N.D.
9	Droogmansia godefroyana *(Kuntze) Schindl*	N.D.	0.04*	N.D.		N.D.	0.18*
		0.19*	N.D.	0.29*		0.29*	N.D.
		N.D.	N.D.	N.D.		0.69*	N.D.
		N.D.	N.D.	N.D.		0.76*	N.D.
		N.D.	N.D.	N.D.		0.89*	N.D.

Table 1 (Continued)

No.	Vegetable Sample	R_f					
		Acetone: MeOH = 1: 1		Acetone: MeOH = 4: 1		Acetone 100%	
		254 nm	366 nm	254 nm	366 nm	254 nm	366 nm
10	*Strychnos minor* Dennst	0.04*	0.04*	N.D.	N.D.	0.01*	N.D.
		N.D.	0.21*	N.D.	N.D.	N.D.	N.D.
		N.D.	N.D.	N.D.	0.83*	N.D.	N.D.
		N.D.	N.D.	N.D.	0.90*	N.D.	N.D.
11	*Cassia tora* Linn.	0.08*	0.08*			0.07*	N.D.
		0.28*	0.28*	Tail*		N.D.	N.D.
		N.D.	0.82*			N.D.	0.90*
12	*Asparagus racemosus* willd.	N.D.	0.13*	0.20*	N.D.	0.03*	N.D.
		N.D.	0.80*	N.D.	0.83*	N.D.	N.D.
		N.D.	N.D.	N.D.	0.90*	N.D.	N.D.
13	*Aganosma marginata* G. Don	N.D.	0.13*	Tail*		0.07*	N.D.
		0.65•	0.65•			N.D.	N.D.
14	*Kaempferia galang* Linn.	0.12•	0.84*	Tail*		0.34*	N.D.
15	*Pluchea indica* Less	N.D.	N.D.	N.D.	0.17*	N.D.	N.D.
		0.23*	0.23*	0.35*	N.D.	N.D.	N.D.
		N.D.	0.86*	N.D.	N.D.	N.D.	N.D.
16	*Blumea sp.*	0.83*	0.83*	N.D.	0.85*	0.04*	N.D.
		N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
17	*Monihoty esculenta* Crantz	0.12*	N.D.	0.05*	0.05*	0.02*	N.D.
		N.D.	N.D.	0.95*	0.95*	N.D.	N.D.
18	*Momordica sp.*	0.11*	0.11*	N.D.	0.09*	0.15*	N.D.
		N.D.	0.37*	0.26*	N.D.	N.D.	N.D.
		N.D.	0.90*	0.87*	0.87*	N.D.	N.D.

Note * is antioxidant spot
• is alkaloid spot
N.D. is non-detected

In the preparative chromatography study (silica gel plate thickness 0.75 mm, mobile phase; acetone : methanol = 3 :1), *Solanum nigrum* Linn., *Phyllanthu amarus* Schumach.& Thonn., and *Blumea sp*. showed more than one spots active components after spraying with DPPH as shown in Table 2.

Table 2 Preparative chromatography R_f values of 4 types of vegetable, *Solanum nigrum* Linn., *Phyllanthus amarus* Schumach.& Thonn., *Blumea sp*., and *Monihoty esculenta* Crantz.

No.	Name	*R_f value* (Mobile phase Acetone: MeOH= 1: 1)	
		254 nm	366 nm
1	*Solanum nigrum* Linn.	0.04*	0.04*
		0.11*	0.11*
		0.14*	0.14*
		N.D	0.19
		N.D	0.24
		N.D	0.30
		N.D	0.67*
		0.85*	0.85*
2	*Phyllanthus amarus* Schumach.& Thonn.	0.09*	N.D
		0.31*	0.31*
		N.D	0.45*
		0.79*	0.79*
		N.D	0.85*
3	*Blumea napifolia* DC.	0.15*	N.D
		0.22*	0.22*
		0.85*	0.85*
4	*Emilia sonchifolia* (Linn.) DC.	0.03*	0.03*
		0.16*	0.16*
		N.D.	0.64
		N.D.	0.79
		0.86	0.86
		0.92*	0.92*

Note * is antioxidant spot
N.D. is non-detected

These results suggest that 17 types of vegetable from Northeastern Thailand have active antioxidant compounds. Some kinds of vegetable have the same R_f value which probably contain the same antioxidant. Organic solvents in particular ratio therefore acted as a mobile phase for the isolation each natural antioxidant from 17 types of vegetable. Moreover, *Blumea sp*. extract (2.91 g) was recrystallized and collected as a white solid (9.5 mg, 0.33%) which showed active spot in DPPH with the R_f of 0.23 (mobile phase; methanol : water = 9 :1, silica gel plate thickness 0.2 mm).

Therefore, natural antioxidant of vegetables from Northeastern Thailand may be further studied its antioxidant activity and biological functions. However, their antioxidant propertied showed be compared to well-known antioxidants, BHT, vitamin E, Gallic acid. The method to evaluate the *in vitro* antioxidant activity may

use any method such as electrochemical method which successfully employed for the direct, rapid, and reliable monitoring of antioxidant activity in herb extracts (Cosio *et. al*., 2005).

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