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# Antioxidant and Antibacterial Activities of Vegetables and Fruits Commonly Consumed in Thailand

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Abstract: The vegetables and fruits commonly consumed in Thailand have been suggested as rich sources of beneficial phytochemicals. In this study, vegetables and fruits native to the Northeast region of Thailand were evaluated for antioxidant and antibacterial activities. For vegetables, the extracts of Limnophila aromatica (Lamk.) Merr. (extracted by 80% ethanol) and Sauropus androgymus (Linn.) Merr. (extracted by distilled water) were found to have significant levels of antioxidant activities measured by DPPH scavenging (IC<sub>so</sub> at 4.92±0.01 and 4.71±0.01 mg mL<sup>-1</sup>, respectively) and FRAP antioxidant assays (74.38±0.25 and 74.00±0.04 mmol FeSO<sub>4</sub> g<sup>-1</sup> of dry weight of vegetable, respectively) and also total phenolic compounds (10.96±0.02 and 10.34±0.01 mg gallic acid eq g<sup>-1</sup> of dry weight of vegetable). For fruits, the extracts derived from Terminalia chebula Retz. when using distilled water and 80% ethanol as solvents were shown to have significantly high levels of DPPH scavenging (IC<sub>50</sub> at 3.73±0.01 and 3.81±0.01 mg mL<sup>-1</sup>, respectively), FRAP antioxidant activities (80.85±0.10 and 65.93±0.11 mmol FeSO<sub>4</sub> g<sup>-1</sup> of dry weight of fruit, respectively) and total phenolic compounds (13.10±0.06 and 10.66±0.02 mg gallic acid eq g<sup>-1</sup> of dry weight of fruit, respectively). The antibacterial assays showed that Moringa oleifera Lam., Limnophila aromatica (Lamk.) Merr., Terminalia chebula Retz. and Phyllanthus emblica Linn. that were extracted using 80% ethanol as solvent were found to have antibacterial activities Staphylococcus aureus, Straphylococcus epidermidis, Streptococcus Propionibacterium acnes. The results in this study may be useful for future application of edible plants that are native to Thailand to be used as cosmetic or therapeutic products.

Key words: Antioxidant activity, antibacterial activity, vegetable extract, fruit extract, Thailand

### INTRODUCTION

Plants are rich sources of several important bioactive compounds. Apart from medicinal herbs, some commonly consumed vegetables and fruits may also have important phytochemicals with pharmacological properties, for example, Brassica vegetables (Podsedek, 2007) and Lycium barbarum fruit (Goji) (Amagase and Farnsworth, 2011). Furthermore, some native vegetables, especially leafy green vegetables and fruits with dark colors have also been found to have bioactive compounds with antioxidant and antimicrobial activities (Andarwulan et al., 2010; Tan et al., 2011; Murillo et al., 2012). The antioxidant activities and bioactive compounds in dietary plants can provide additional health benefits for prevention of cardiovascular disease, cancer, neurological decline and diabetes (Wootton-Beard and Ryan, 2011). In Thailand, a variety of native vegetables and fruits that are used as daily food and seasoning are proven to have high antioxidant activities in addition to their nutritional values, for instance, Cassia siamea, Tagetes erecta, Antigonon leptopus, Gymnema inodorum, Piper sarmentosum,

Mentha arvensis, Diospyros decandra, Terminalia chebula and Phyllanthus emblica (Chanwitheesuk et al., 2005; Kaisoon et al., 2011; Kubola et al., 2011). There are a large number of Thai vegetables and fruits that are attractive due to their health-related functional properties as well as their possible future application for prevention and therapeutic purposes. However, there are many of them that remain unexplored. Therefore, this study aims to evaluate the antioxidant and antibacterial activities of the vegetables and fruits commonly consumed in the Northeast region of Thailand.

# MATERIALS AND METHODS

Preparation of plant extracts: The vegetables and fruits that are commonly consumed in the Northeast region of Thailand were collect or purchased from Maha Sarakham provinces. The vegetables (their common and Thai names are written in the brackets, respectively) included Neptunia oleracea Lour. (water mimosa, Krashednam), Limnophila aromatica (Lamk.) Merr. (rice paddy herb, Kayang), Coccinia grandis (L.) J. Voigt (ivy gourd, Tamleung), Basella alba Linn. (Ceylon spinach,

Plungkhaw), Sauropus androgynus (Linn.) Merr. (sweet leaf bush, Wanban) and Moringa oleifera Lam. The fruits included (drumstick tree, Maroom). Flacourtia indica (Burm. f.) Merr. (governor's plum, Takhobpa), Phyllanthus emblica Linn. (Indian gooseberry, Makhampom), Phyllanthus acidus (L.) Skeels. (star gooseberry, Mayom), Antidesma Müll. Arg. thw aitesianum (Mao, Mamaoluang), Ziziphus oenoplia (L.) Mill. (jackal jujube, Lepmeaw) and Terminalia chebula Retz. (black myrobalan, Smore). These vegetables (leaves, stems and branches) and fruits (peels without seeds) were air dried to have less than 10% humidity, ground into fine powder and extracted using distilled water (DW) and 80% ethanol (E80) as solvents at the ratio 1:5 w/v. The extraction process was performed for 12 h with agitation at 150 rpm at room temperature (28-30°C). The resulting solutions were centrifuged at 4000 rpm for 10 min and the supernatants were concentrated using vacuum rotary evaporator at 45°C. The percentage of extraction yield was calculated as following:

$$Yield (\%) = \frac{Weight of extract (g)}{Weight of dried vegetable (g)} \times 100$$

Antioxidant activity determination by DPPH scavenging assay: DPPH scavenging assay for evaluation of antioxidant activities was described previously (Butsat and Siriamornpun, 2010). Plant extract (100  $\mu$ L) was mixed with freshly prepared 0.1 mM DPPH° solution (1.9 mL) and incubated at room temperature in the dark for 30 min and then measured for absorbance at 517 nm. BHA (butylated hydroxyamsole) was used as standard reagent. The levels of DPPH scavenging activities of the extracts were calculated and expressed as IC<sub>50</sub> (mg mL<sup>-1</sup>). The experiments were performed in three replicates.

Antioxidant activity determination by ferric reducing ability of plasma (FRAP) assay: FRAP assay for determination of antioxidant activities was previously described by Butsat and Siriamompun (2010). FRAP stock solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution (in 40 mM HCl) and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O solution. FRAP solution (1.7 mL) must be prepared freshly and warmed at 37°C before adding to plant extract (300  $\mu$ L). The mixture was incubated at room temperature for 1 h and then measured for absorbance at 593 nm. The standard curve was plotted using the result obtained from FeSO<sub>4</sub> standard reagent. The antioxidant activities of the extracts were calculated and expressed as mol FeSO<sub>4</sub> eq g<sup>-1</sup> of dry weight of vegetable or fruit. The experiments were carried out in three replicates.

Total phenolic compound measurement: The level of total phenolic compounds was measured by Folin-Ciocalteu method as previously described (Butsat and Siriamornpun, 2010). Plant extract (200 μL) was mixed with Folin-Ciocalteu reagent (diluted 1:10 with sterile distilled water) (1 mL), shaken for 1 min and added with 10% Na<sub>2</sub>CO<sub>3</sub> (800 µL). After that, the mixture was topped up with sterile distilled water to have total volume of 5 mL and incubated at room temperature in the dark for 2 h. Finally, the mixture was measured for absorbance at 760 nm. Gallic acid was used as standard reagent. The levels of total phenolic compounds of the extracts were calculated and expressed as mg gallic acid eq g-1 of dry weight of vegetable or fruit. The experiments were performed in three replicates.

Antibacterial activity evaluation by agar well diffusion assay: Agar well diffusion assay for determination of antibacterial activity was performed as described previously (Oke et al., 2009) with some modifications. Staphylococcus aureus, Straphylococcus epidermidis, Streptococcus pyogenes and Propionibacterium acnes were grown overnight at 37°C and adjusted to approximately 1×10<sup>8</sup> CFU mL<sup>-1</sup>. The bacterial culture (100 µL) was then spread onto Mueller Hinton Agar (MHA) plate and let to dry for 10 min before being punctured by 0.6 cm sterile cork borer. Plant extract (30 µL) was added to each agar well and the plate was incubated at 37°C overnight. The antibacterial activities of the extracts were measured by the diameter size of inhibition zone around the agar well.

### RESULTS

Levels of antioxidant activities and total phenolic **compounds:** The vegetables extracts were shown to have antioxidant activities measured by DPPH scavenging and FRAP assays (Table 1). The extracts of N. oleracea (extracted by DW and E80), L. aromatica (DW and E80), C. grandis (DW) and S. androgynus (DW) had significantly high antioxidant activities measured by DPPH scavenging assay. However, only the extracts of L. aromatica (E80) and S. androgynus (DW) that were also found to have significant levels of antioxidant activities measured by FRAP assays (74.38±0.25 and 74.00±0.04 mmol FeSO<sub>4</sub> eq g<sup>-1</sup> of dry weight of vegetable, respectively) and total phenolic compounds  $(10.96\pm0.02 \text{ and } 10.34\pm0.01 \text{ mg gallic acid eq g}^{-1} \text{ of }$ dry weight of vegetable, respectively). Interestingly, B. alba extract (DW) that had the highest FRAP antioxidant activities (80.59±0.09 mmol FeSO<sub>4</sub> eq g<sup>-1</sup> of dry weight of vegetable) was found to have rather

Table 1: Antioxidant activities and total phenolic compounds of vegetable extracts

		DPPH IC <sub>50</sub>	FRAP antioxidant activity	Total phenolic compounds
Vegetable	Solvent	$(mg mL^{-1})$	(mmol FeSO <sub>4</sub> eq g <sup>-1</sup> dried vegetable)	(mg gallic acid eq g <sup>-1</sup> dried vegetable)
Neptunia oleracea Lour.	DW	$4.34\pm0.28^{ab}$	27.62±0.05g	4.16±0.04 <sup>f</sup>
	E80	$4.68\pm0.03^{ab}$	29.31±0.07 <sup>f</sup>	$4.84\pm0.00^{\rm ef}$
Limnophila aromatica (Lamk.) Merr.	DW	4.23±0.01 <sup>a</sup>	34.71±0.04e	$5.61 \pm 0.01^{de}$
	E80	$4.92\pm0.01^{ab}$	$74.38 \pm 0.25^{b}$	$10.96\pm0.02^{a}$
Coccinia grandis (L.) J. Voigt	DW	$4.39\pm0.01^{ab}$	$68.06\pm0.08^{\circ}$	9.96±0.01 <sup>b</sup>
	E80	$5.22\pm0.03^{b}$	25.38±0.59 <sup>h</sup>	3.15±0.01 <sup>g</sup>
Basella alba Liun.	DW	6.71±0.04°	$80.59\pm0.09^a$	$8.18\pm0.03^{\circ}$
	E80	$11.05\pm0.13^{d}$	$24.52\pm0.04^{i}$	$3.21\pm0.01^{g}$
Sauropus androgynus (Liun.) Merr.	DW	$4.71\pm0.01^{ab}$	$74.00\pm0.04^{b}$	$10.34\pm0.01^{ab}$
	E80	$6.30\pm0.04^{\circ}$	$37.51\pm0.05^{d}$	5.99±0.91 <sup>d</sup>
Moringa oleifera Lam.	DW	nd	$8.35\pm0.55^{j}$	$0.36\pm0.01^{h}$
	E80	77.97±0.92°	$5.77\pm0.01^{k}$	$0.22\pm0.02^{h}$

Values (Mean±SD) in the same column with the same lower-case letter(s) are not significant different as determined by Duncan's test at p<0.05, DW: Distilled water, E80: 80% ethanol, nd: Not detected

Table 2: Antioxidant activities and total phenolic compounds of fruit extracts

Fruit	Solvent	DPPH IC <sub>50</sub> (mg mL <sup>-1</sup> )	FRAP antioxidant activity (mmol FeSO <sub>4</sub> eq g <sup>-1</sup> dried fruit)	Total phenolic compounds (mg gallic acid eq g <sup>-1</sup> dried fruit)
Flacourtia indica (Burm. f.) Merr.	DW	4.38±0.12 <sup>b</sup>	65.14±0.07°	5.12±0.02°
` '	E80	$7.34\pm0.30^{d}$	$60.67\pm0.09^{d}$	$4.55\pm0.03^{\rm f}$
<i>Phyllanthus emblica</i> Linn.	DW	3.48±0.03 <sup>a</sup>	46.18±0.05°	7.72±0.03°
-	E80	$3.88 \pm 0.02^{ab}$	$38.95\pm0.07^{f}$	$6.26 \pm 0.01^{d}$
Phyllanthus acidus (L.) Skeels.	DW	10.93±0.56°	$6.51\pm0.03^{k}$	$0.75\pm0.01^{j}$
	E80	10.41±0.15°	$5.02\pm0.05^{1}$	$0.66\pm0.01^{k}$
Antidesma thwaitesianum Müll. Arg.	DW	5.07±0.03°	33.02±0.05 <sup>g</sup>	$4.52\pm0.01^{\rm f}$
	E80	$7.38\pm0.22^{d}$	$28.60\pm0.11^{i}$	2.80±0.01 <sup>g</sup>
Ziziphus oenoplia (L.) Mill.	DW	$15.35\pm0.28^{f}$	$31.54\pm0.23^{h}$	$2.72\pm0.01^{h}$
	E80	31.47±0.31g	26.73±0.04 <sup>j</sup>	$2.07\pm0.01^{i}$
Terminalia chebula Retz.	DW	$3.73\pm0.01$ ab	$80.85\pm0.10^{a}$	$13.10\pm0.06^{a}$
	E80	$3.81\pm0.01^{ab}$	65.93±0.11 <sup>b</sup>	$10.66\pm0.02^{b}$

Values (Mean±SD) in the same column with the same lower-case letter(s) are not significant different as determined by Duncan's test at p<0.05, DW: Distilled water, E80: 80% ethanol, nd: Not detected

moderate levels of DPPH IC<sub>50</sub> at  $6.71\pm0.04$  mg mL<sup>-1</sup> and total phenolic compounds at  $8.18\pm0.03$  mg gallic acid eq g<sup>-1</sup> of dry weight of vegetable.

The fruits extracts that were found to have significantly high levels of both antioxidant activities and total phenolic compounds are those derived from *T. chebula* (extracted by DW and E80) which giving DPPH IC<sub>50</sub> at 3.73±0.01 and 3.81±0.01 mg mL<sup>-1</sup>, FRAP antioxidant activities at 80.85±0.10 and 65.93±0.11 mmol FeSO<sub>4</sub> g<sup>-1</sup> of dry weight of fruit and total phenolic compounds at 13.10±0.06 and 10.66±0.02 mg gallic acid eq g<sup>-1</sup> of dry weight of fruit, respectively (Table 2). Also, *P. emblica* extracts (DW and E80) were shown to have significant levels of DPPH IC<sub>50</sub> at 3.48±0.03 and 3.88±0.02 mg mL<sup>-1</sup>, respectively, but rather moderate levels of FRAP antioxidant activities and total phenolic compounds.

Antibacterial activities: For vegetable extracts, only *M. oleifera* and *L. aromatica* extracts were found to have antibacterial activities against all four tested bacteria (Table 3). The highest antibacterial activities was found in the E80 extract of *M. oleifera* that could inhibit *S. aureus*, *S. epidermidis*, *S. pyogenes* and *P. acnes* by producing

inhibition zones with the diameters of  $1.50\pm0.12$ ,  $1.57\pm0.18$ ,  $1.50\pm0.23$  and  $2.50\pm0.27$  cm, respectively, followed by the E80 extract of L. aromatica that produced inhibition zone at  $1.20\pm0.06$ ,  $1.20\pm0.12$ ,  $0.93\pm0.07$  and  $1.27\pm0.12$  cm, respectively. Moderate inhibition activities were found in the DW extract of M. oleifera which producing zone at  $1.03\pm0.43$ ,  $1.10\pm0.50$ ,  $1.17\pm0.57$  and  $1.53\pm0.48$  cm, respectively.

For fruit extracts, inhibition of all four tested bacteria were found when applying with the extracts of T. chebula (E80) that producing clear zones against S. aureus, S. epidermidis, S. pyogenes and P. acnes at  $1.37\pm0.03$ ,  $1.57\pm0.03$ ,  $1.23\pm0.12$  and  $1.68\pm0.06$ , respectively (Table 4). Moreover, the extracts of P. emblica (E80) could also inhibit these four bacteria by inducing clear zones at  $1.68\pm0.06$ ,  $1.59\pm0.05$ ,  $1.23\pm0.12$  and  $1.68\pm0.06$ , respectively.

Streptomycin (10 IU mL<sup>-1</sup>) was used as positive control group and inhibited *S. aureus*, *S. epidermidis*, *S. pyogenes* and *P. acnes* by producing clear zone at 3.47±0.15, 1.57±0.07, 1.83±0.03 and 1.63±0.07 cm, respectively. The solvents, DW and E80, were used as negative control groups and they did not show any inhibition against these four bacteria.

Table 3: Antibacterial activities of vegetable extracts

Vegetables		Inhibition zone (cm)				
	Solvent	S aureus	S. epidermidis	S pyogenes	P. acnes	
Neptunia oleracea Lour.	DW	-		-	-	
	E80	-		-	$0.73\pm0.03$	
Limnophila aromatica (Lamk.) Мет.	DW	-	-	-	-	
	E80	$1.20\pm0.06$	$1.20\pm0.12$	$0.93 \pm 0.07$	$1.27\pm0.12$	
Coccinia grandis (L.) J. Voigt	DW	-	-	-	-	
	E80	-	-	-	-	
<i>Basella alba</i> Linn.	DW	-	-	-	-	
	E80	-	-	-	-	
Sauropus androgynus (Linn.) Merr.	DW	-	-	-	-	
	E80	-	-	-	-	
Moringa oleifera Lam.	DW	$1.03\pm0.43$	$1.10\pm0.50$	1.17±0.57	1.53±0.48	
	E80	$1.50\pm0.12$	1.57±0.18	1.50±0.23	2.50±0.27	

The size of cork borer is 0.6 cm

Table 4: Antibacterial activities of fruit extracts

Fruit		Inhibition zone (cm)				
	Solvent	S. aureus	S epidermidis	S. pyogenes	P. acnes	
Flacourtia indica (Burm.f.) Merr.	DW	-		-	-	
	E80	-	-	-	$1.03\pm0.07$	
Phyllauthus emblic a Linn.	DW	1.20±0.06	$1.20\pm0.06$	$1.00\pm0.00$	1.30±0.06	
	E80	$1.68\pm0.06$	$1.59\pm0.05$	$1.23\pm0.12$	1.68±0.06	
Phyllauthus acidus Skeels	DW	-	-	-	-	
	E80	-	-	-	-	
Antidesma thwaitesianum Muell. Arg	DW	-	-	-	-	
	E80	-	-	-	$1.10\pm0.21$	
Ziziphus oenoplia (L.) Mill	DW	-	-	-	-	
	E80	-	-	-	-	
Terminalia chebula Retz.	DW	$1.23\pm0.03$	$1.20\pm0.06$	$1.00\pm0.00$	1.30±0.06	
	E80	$1.37\pm0.03$	$1.57\pm0.03$	$1.23\pm0.12$	$1.68\pm0.06$	

The size of cork borer is 0.6 cm

# DISCUSSION

In this study, the extracts of vegetables, L. aromatica, S. androgynus and B. alba, were shown to have good antioxidant activities. The phenolic compounds (especially flavonoids) and essential oils in the vegetables extracts have been indicated to strongly promote extracts' antioxidant activities (Guimaraes et al., 2010). In this study, high levels of total phenolic compounds in the extract of S. androgynus (DW) could play an important role on promoting its antioxidant activities. The previous report suggested that the extract of Indonesian S. androgynus (extracted by 95% ethanol) was found to have high level of total phenolic compounds at 1.49±0.15 mg gallic acid eq g-1 fresh weight (Andarwulan et al., 2010). Moreover, the extract of L. aromatica (E80) was found to have strong aroma and thus could be assumed that it may contain high quantity of essential oil which may contribute to its high antioxidant activities. In addition, the ethanolic extract of B. alba leaves was reported to have robust antioxidant properties and provide strong protection against chemical carcinogenesis induced hepatocarcinogenesis in animal model (Reddy et al., 2008).

The extracts of T. chebula fruit were shown to have very strong antioxidant activities and high levels of total phenolic compounds. Recent reports have suggested that an antioxidant protein (~16 kDa) from T. chebula fruit has been successfully purified and characterized and it may be a major compound responsible for antioxidant activities of T. chebula fruit (Srivastava et al., 2012). Also, there are a number of previous reports that showed the strong antioxidant properties and high levels of phenolic compounds of the extracts derived from T. chebula fruit (Pfundstein et al., 2010; Saleem et al., 2002; Nampoothiri et al., 2011; Tubtimdee and Shotipruk, 2011). These results hence supported the findings of strong antioxidant activities and high levels of total phenolic compounds of T. chebula fruit extracts in this study. In addition, although P. emblica extracts was found to have slightly lower FRAP antioxidant activities and total phenolic compounds, they were found to have strong DPPH IC<sub>50</sub> similar to previous reports (Liu *et al.*, 2008a,b; Luo et al., 2009, 2011).

This study showed that the E80 extracts of M. oleifera, L. aromatica, T. chebula and P. emblica were found to have antibacterial activities against Staphylococcus aureus, Straphylococcus epidermidis,

Streptococcus pyogenes and Propionibacterium acnes. As the negative control group using 80% ethanol alone showed no inhibition against these four bacteria, it is therefore unlikely that the residual ethanol in plant extracts could have any effect on their antibacterial activities. Furthermore, the antibacterial effects of these vegetable and fruits extracts have been previously reported. The extracts of M. oleifera were shown to inhibit Salmonella typhi, Pseudomonas aeruginosa and S. aureus (Doughari et al., 2007; Caceres et al., 1991). Also, the nanoparticles that applied with phenolic compounds (tannins) derived from T. chebula aqueous extract were shown to be effective at inhibiting S. aureus and Escherichia coli (Kumar et al., 2012). Moreover, the water extract of T. chebula was found to contain a heat stable agent(s) that could strongly Helicobacter pyroli (Malekzadeh et al., 2001). The extracts of P. emblica were also previously found to inhibit S. aureus due to a variety of its phenolic compounds (Mayachiew and Devahastin, 2008).

#### CONCLUSION

In this study, the antioxidant and antibacterial activities of Thai local vegetables and fruits have been illustrated. The results in this study may suggest the potentials of the extracts derived from *S. androgynous*, *L. aromatica*, *B. alba*, *M. oleifera*, *T. chebula* and *P. emblica* for application in cosmetic and/or therapeutic products due to their high levels of antioxidant activities and/or total phenolic compounds. These edible plants may be used as alternative to synthetic chemicals particularly because of their safety and ample amount in many local areas of Thailand.

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