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Antioxidant Activity, Total Phenolics and Isoflavones in Vegetables Available in Thailand

Ratana Sapbamrer [1]*, Ittirit Moonmuang [2] & Piyawan Nuntaboon [2]

[1] *School of Medicine, University of Phayao, , 56000 Phayao, Thailand*
[2] *School of Medical Sciences, University of Phayao, 56000 Phayao, Thailand*

ABSTRACT

Introduction: Increased interest in phenolic compounds is largely due to findings of their association with antioxidant, antimutagenic, antibacterial, anticarcinogenic, and anti-inflammatory activities with reduced risk of free radicals related diseases. Local vegetables of Thailand were examined for antioxidant activity, total phenolics and isoflavone contents. **Methods:** Thirty edible leaf and 13 other-parts of vegetable plants were collected from the markets in Northern Thailand for analysis of antioxidant activity (DPPH and ABTS assays), total phenolics, and isoflavones. **Results:** The antioxidant activity for DPPH assay and total phenolics of edible leaf vegetables (EC_{50} = 541.2±498.9 μg/mL and 2438.7±3342.7 μg GAE/g dry extract respectively) were significantly higher than those of the other edible plant parts (EC_{50} = 1315.5±1303.4 μg/mL and 1263.3±3281.7 μg GAE/g dry extract respectively). Ten types of edible leaf vegetables and only one example of plant part, namely ginger, exhibited high antioxidant activity. The antioxidant activities for DPPH and ABTS assays were associated with total phenolics concentration. **Conclusion:** Antioxidant activity and total phenolics of Thai edible leaf vegetables were higher than those of other edible plant parts. The Thai copper pod showed the highest levels of total phenolics and isoflavones, and strong antioxidant activity. Further investigation should be undertaken to examine the active mechanisms of these properties in relations to health benefits.

Key words: ABTS, antioxidant, DPPH, herbs, isoflavone, total phenolics, vegetables

INTRODUCTION

Phenolic compounds are found naturally in several types of plants such as vegetables, fruits, cereals, and herbs. There is increased interest in phenolic compounds because of their antioxidant, antimutagenic, antibacterial, anticarcinogenic, and anti-inflammatory activities (Oviasogie, Okoro & Ndiokwere, 2009; Wootton-Beard, Moran & Ryan, 2011). Furthermore, isoflavones are categorised into the flavonoid class which is in phenolic compounds (Cornwell, Cohick & Raskin, 2004; Corradini *et al.*, 2011). Their remarkable properties also include antioxidant and estrogenic activities (Cederroth *et al.*, 2010; Patel *et al.*, 2001). Epidemiological studies have demonstrated that dietary plants rich in phenolic compounds and antioxidants resist free radicals-related diseases such

* Correspondence: Ratana Sapbamrer; Email: *lekratana56@yahoo.com*

as cancers, rheumatoid, Alzheimer, and cardiovascular disorders (Heber, 2004). Similarly, several studies have shown that dietary isoflavones are associated with beneficial health effects such as decreased incidence of breast and reproductive cancers and coronary heart disease, and a decrease in cholesterol level (Bandera *et al.*, 2011; Hedelin *et al.*, 2006; Ozasa *et al.*, 2004; Sapbamrer, Visavarungroj & Suttajit, 2013). Therefore, it is suggested that antioxidant activities of total phenolics and isoflavones result in multiple health benefits.

Although antioxidants and total phenolics are found in both vegetables and fruits, daily intake of vegetables is greater than fruits because of their cheap price and availability (Deng *et al.*, 2013). There are several types of local vegetables in Thailand; most are edible leaf vegetables and other edible plant parts include the root, fruit, flower, and sheath. They are an important source of food for Thai people because they are cheap and easy to buy or cultivate. There is a dearth of research on antioxidant activity and total phenolics of Thai vegetables. In addition, data on the correlations among antioxidant activities, total phenolics, and isoflavones in vegetables are rather scarce. Thus, the objectives of this study were (1) to determine antioxidant activity, total phenolics, and isoflavones in local vegetables in Thailand; (2) to compare antioxidant activity, total phenolics, and isoflavones in edible leaf and other-part vegetables; and (3) to investigate correlations among antioxidant activity, total phenolics, and isoflavones of these local vegetables.

METHODS

Types of vegetables

Forty-three local vegetables in Thailand were purchased from the markets in Northern Thailand; they consisted of 30 edible leaf and 13 other-part vegetables (root, fruit, flower, and sheath). They were identified botanically by BGO Plant Databases, the Botanical Garden Organisation, Thailand. Common names, scientific names, and edible parts of vegetables are shown in Tables 1 and 2.

Sample preparation and extraction

An amount of 5 kg of each vegetable was purchased; only the edible parts of the vegetable were selected through stepwise sampling until the final sample stood at 500 g. The sample was chopped and dried in a hot air oven at 55°C for 16 hours. Finally, the sample was ground into powder by using a grinder, and stored at -20°C until extraction.

Sample extraction for antioxidant activity and total phenolics determination was modified from the method of Shinde, Malpathak & Fulzele (2010). One gram of dried sample was mixed with 40 mL of methanol, sonicated (33 Kilohertz and 40°C) for 40 minutes, filtered with filter paper (Whatman no.1), and evaporated at 45°C. Finally, the extract was dissolved with 5 mL of methanol, and stored at -20°C until analysis.

Sample extraction for isoflavone analysis was performed according to the method of Nakamura *et al.* (2000). Five grams of dried sample were placed in a centrifuge tube. One mL of internal standard solution (flavone 210 μg), 10 mL of 10 N HCL solution, and 40 mL of 0.05% BHT solution were added to the centrifuge tube and sonicated for 30 min. The tube was cooled and centrifuged at 1000×g for 20 min at 5°C, and the volume was adjusted to 50 mL with ethanol. The sample solution was cleaned-up using C18 ODS cartridge (Agilent, USA) before HPLC analysis. Fifty mL of each sample solution was applied to the cartridge column preconditioned with 2 mL of 20% methanol and 20 mL of water. Isoflavones and internal standard were eluted with exactly 1 mL of the methanol. All solvents used in the experiment were analytical grade solvents (JT Baker, USA).

Table 1. Common name, scientific name, family of edible leaf vegetables

Common name	*Scientific name*	Family
Bamboo grass	*Tiliacoratriandra*	Menispermaceae
Pak-wan*	*Sauropus androgynous*	Euphorbiaceae
Thai copper pod	*Cassia siamea*	Leguminosae
Lead tree	*Leucaenaleucocephala*	Leguminosae
Vietnamese coriander	*Polygonumodoratum*	Polygonaceae
Pak-herd*	*Fiscuslacor*	Moraceae
Kitchen mint	*Menthacordifolia*	Labiatae
Horapha-chang*	*Ocimumgratissimum*	Labiatae
Ivy gourd	*Cocciniagrandis*	Cucurbitaceae
Indian borage	*Plectranthusamboinicus*	Lamiaceae
Hairy basil	*Ocimumcanum*	Labiatae
Gurma	*Gymnemainodorum*	Asclepiadaceae
Pak-saw*	*Marsdeniaglabra*	Asclepiadaceae
Ming aralia	*Polysciasfruticosa*	Araliaceae
Orchid tree	*Bauhinia purpurea*	Leguminosae
Chinese lizard tail	*Houttuyniacordata*	Saururaceae
Sweet basil	*Ocimumbasilicum*	Labiatae
Asiatic pennywort	*Centellaasiatica*	Umbelliferae
Tooth-ache plant	*Spilanthesacmella*	Asteraceae
Chinese cabbage	*Brassica chinensis*	Cruciferae
Sawtooth coriander	*Eryngiumfoetidum*	Apiaceae
Soap pod	*Acacia concinna*	Leguminosae
Rat-tailed radish	*Raphanussativus*	Brassicaceae
Malabar spinach	*Basella alba*	Basellaceae
Vegetable fern	*Diplaziumesculentum*	Athyriaceae
Horse radish tree	*Moringaoleifera*	Moringaceae
Pak-nam*	*Lasiaspinosa*	Araceae
Holy basil	*Ocimumtenuiflorum*	Labiatae
Cha-om*	*Acacia pennata*	Leguminosae
Golden shower	*Cassia fistula*	Caesalpinioideae

* Vegetable name in Thai

Table 2. Common name, scientific name, family of edible parts of vegetable plants

Common name	*Scientific name*	*Family*	*Edible plant parts*
Ginger	*Zingiberofficinale*	Zingiberaceae	root
Plate brush eggplant	*Solanumtorvum*	Solanaceae	Fruit
Jackfruit	*Artocarpusheterophyllus*	Moraceae	Fruit
Broken bones tree	*Oroxylumindicum*	Bignoniaceae	Sheath
Vegetable humming bird	*Sesbaniagrandiflora*	Fabaceae	Flower
Chilli pepper	*Capsicum annum*	Solanaceae	Fruit
Cowslip creeper	*Telosma minor*	Asclepiadaceae	Flower
Baa-kwaeng-kom*	*Solanumindicum*	Solanaceae	Fruit
Bitter cucumber	*Momordicacharantia*	Cucurbitaceae	Fruit
Angled gourd	*Luffaacutangula*	Cucurbitaceae	Fruit
Galangal	*Alpiniagalanga*	Zingiberaceae	Root
Banana	*Musa Sapientum*	Musaceae	Flower
Salaer*	*Broussonetiakurreii*	Moraceae	Flower

* Vegetable name in Thai

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

Antioxidant activity for DPPH assay was determined by modifying the method of Katsube *et al.* (2004). The extract was diluted with water stepwise and 20 μL of dilution was pipetted into a 96-well plate. One hundred and eighty μL of DPPH solution dissolved in 50% ethanol solution was added to each well. The plate was shaken for 5 minutes at room temperature. The absorption at 540 nanometers (nm) was measured by microplate reader (MultiRead 400, Anthos). The correlation factor of the calibration curve (R^2) was 0.997. The radical scavenging activity was expressed as the median effective concentration (EC_{50}, μg/mL).

ABTS (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging assay

Antioxidant activity for ABTS assay was determined by modifying the method of Re *et al.* (1999). The $ABTS^{+}$cation radicals were produced by the reaction between 7 nM of ABTS in water and 2.45 nM of potassium persulfate, and stored in the dark at room temperature for 12 hours. The solution was diluted with phosphate buffer to get an absorbance of 0.70 ± 0.02 at 734 nm before using. Free radical scavenging activity was determined by mixing 10 μL of the extract (1 mg/mL) with ABTS working standard. The change in absorbance was measured after 6 min. The correlation factor of the calibration curve (R^2) was 0.999. The radical scavenging activity was expressed as the median effective concentration (EC_{50}, μg/mL).

Determination of total phenolic content

Determination of total phenolics was measured by modifying the method of Kähkönen *et al.* (1999). Two hundred μL of the extract was introduced into the test tube. One mL of Folin-Ciocalteu's reagent and 0.8 mL of sodium carbonate (7.5%) were added. The tube was shaken and allowed to stand for 30 minutes. Absorption at 765 nm was measured by UV-visible spectrophotometer. The total phenolics were expressed as gallic acid equivalents (GAE) in μg/g dry extract. The correlation factor of the calibration curve (R^2) was 0.989.

Determination of isoflavones

Sample analysis was modified from the method of Nakamura *et al.* (2000). The extract was analysed by HPLC (Prostar, Varian). The LC conditions were as follows: Omnisphere C18, 250×4.6 mm column (Varian); mobile phases (A) water–phosphoric acid 1,000 + 1 (v/v) and (B) acetonitrile–water–phosphoric acid 800 + 200 + 1 (v/v/v); linear gradient program, 35°C column oven temperature; UV detector; 10 μL injection volume; and 260 nm monitoring wavelength. Recovery was 90.4% for genistein and 96.2% for daidzein. The quantitation limit was 0.747 μg/mL for genistein and 0.686 μg/mL for daidzein. The intrabatch coefficient of variation (% CV) was 4.78% for genistein and 5.34% for daidzein. The interbatch % CV was 7.52% for genistein and 7.15% for daidzein. The isoflavones were expressed as μg/g dry weight of vegetable. The correlation factor of the calibration curve (R^2) was 0.987 for genistein and 0.992 for daidzein.

Statistical analysis

All experiments, including EC_{50} for DPPH and ABTS assays, total phenolics, and isoflavones, were determined in triplicate, and presented in the form of statistical means and standard deviation (SD.). The variables with non-normal distribution were natural logarithms transformed before the parametric test. Data were analysed using Student's *t*-test for the difference in antioxidant activity, total phenolics, and isoflavones between edible leaf and other-part vegetables, and Spearman rank correlation coefficient for the correlation among the analysed parameters.

RESULTS

Antioxidant activity, total phenolics, and isoflavones in vegetables

Table 3 and Table 4 present antioxidant activity, total phenolics, and isoflavones in edible leaf and other-plant parts. The highest antioxidant activity for DPPH assay in edible leaf vegetables was found in bamboo grass (EC_{50} = 16.69±2.27 μg/mL), followed by Pak-wan, Thai copper pod, lead tree, and Vietnamese coriander, respectively. Meanwhile, the highest activity for ABTS assay was found in lead tree (EC_{50} = 7.73±1.61 μg/mL), followed by Vietnamese coriander, Chinese lizard tail, Horapha-chang, and Thai copper pod, respectively.

Among the edible plant parts, the highest activity for DPPH assay was found in ginger (EC_{50} = 277.8±10.96 μg/mL), followed by plate brush eggplant, jackfruit, broken bone tree, and vegetable humming bird, respectively. Meanwhile, the highest activity for ABTS assay was found in ginger (EC_{50} = 12.41±6.55 μg/mL),

Table 3. Antioxidant activity, total phenolics, and isoflavones in edible leaf vegetables

Common name	*Mean ± SD*				
	EC_{50} DPPH (μg/mL)	*EC_{50} ABTS (μg/mL)*	*Total phenolics (μg GAE/g)[a]*	*Daidzein (μg/g)[b]*	*Genistein (μg/g)[b]*
Bamboo grass	16.69 ± 2.27	46.44 ± 8.67	797.6 ± 349.3	0.59 ± 0.51	3.15 ± 0.87
Pak-wan*	35.45 ± 1.85	53.60 ± 1.94	2840.4 ± 4284.5	3.03 ± 0.66	1.28 ± 0.48
Thai copper pod	37.00 ± 4.58	23.06 ± 3.57	14960.9 ± 5067.7	22.53 ± 7.05	20.27 ± 9.47
Lead tree	45.53 ± 6.56	7.73 ± 1.61	4292.6 ± 3280.4	4.15 ± 1.35	1.54 ± 0.66
Vietnamese coriander	47.46 ± 0.25	10.88 ± 3.59	2058.6 ± 796.3	2.21 ± 1.44	0.30 ± 0.30
Pak-herd*	75.91 ± 10.98	45.95 ± 1.26	1585.7 ± 1005.9	13.39 ± 3.80	4.28 ± 0.98
Kitchen mint	153.9 ± 36.47	102.7 ± 6.03	3528.5 ± 673.3	5.9 ± 4.20	0.34 ± 1.67
Horapha-chang*	262.7 ± 26.86	21.26 ± 2.44	4397.9 ± 999.7	7.85 ± 2.87	3.35 ± 0.92
Ivy gourd	389.0 ± 53.59	67.76 ± 4.00	1554.2 ± 898.8	4.58 ± 1.23	0.55 ± 0.08
Indian borage	449.0 ± 48.02	71.34 ± 12.99	858.0 ± 503.3	0.61 ± 0.44	2.91 ± 0.84
Hairy basil	455.5 ± 34.51	81.26 ± 13.81	1717.9 ± 699.6	ND	0.89 ± 0.08
Gurma	474.3 ± 71.23	38.47 ± 5.39	583.0 ± 153.7	0.53 ± 0.41	0.62 ± 0.10
Pak-saw*	486.4 ± 15.15	36.00 ± 4.64	280.7 ± 45.35	9.68 ± 1.37	1.66 ± 0.33
Ming aralia	503.5 ± 52.07	80.09 ± 12.82	11943.2 ± 16227.7	9.83 ± 1.47	4.06 ± 0.86
Orchid tree	700.6 ± 75.62	>100	521.6 ± 204.4	0.61 ± 0.75	0.80 ± 0.92
Chinese lizard tail	788.0 ± 31.54	17.66 ± 7.11	6004.8 ± 3649.4	1.71 ± 0.09	0.1 ± 0.001
Sweet basil	838.7 ± 25.29	28.86 ± 3.05	2672.9 ± 507.7	0.95 ± 0.81	0.53 ± 0.17
Asiatic pennywort	850.7 ± 48.83	201.7 ± 5.21	201.8 ± 50.72	5.12 ± 1.12	1.69 ± 0.35
Tooth-ache plant	976.5 ± 193.3	110.0 ± 28.49	648.7 ± 266.4	2.87 ± 0.77	0.99 ± 0.11
Chinese cabbage	1040.6 ± 174.3	102.4 ± 24.70	484.8 ± 172.9	1.12 ± 0.45	0.42 ± 0.32
Sawtooth coriander	1227.9 ± 21.86	218.8 ± 57.07	453.6 ± 260.0	0.16 ± 0.05	0.39 ± 0.05
Soap pod	2051.0 ± 1268.3	401.7 ± 28.55	696.1 ± 134.3	10.94 ± 3.04	1.93 ± 0.04
Rat-tailed radish	>75	>250	1033.9 ± 843.2	1.88 ± 0.02	12.13 ± 1.63
Malabar spinach	>500	>525	501.0 ± 143.6	1.45 ± 0.41	0.51 ± 0.05
Vegetable fern	>800	>80	2840.1 ± 174.7	0.65 ± 0.40	0.13 ± 0.16
Horse radish tree	>1,000	82.66 ± 12.84	2463.8 ± 1761.4	3.11 ± 0.71	0.38 ± 0.05
Pak-nam*	>2,000	>225	1032.4 ± 467.0	2.32 ± 0.73	0.42 ± 0.22
Holy basil	>3,000	97.52 ± 20.67	997.6 ± 361.0	3.20 ± 0.60	0.64 ± 0.31
Cha-om*	>7,000	504.33 ± 35.87	1187.4 ± 129.6	2.85 ± 0.34	0.45 ± 0.08
Golden shower	>10,250	84.67 ± 10.93	21.75 ± 11.85	0.96 ± 0.39	0.16 ± 0.04

* Vegetable name in Thai; [a] expressed in μg GAE/g dry extract; b expressed in μg/g dry weight of vegetable; ND = not detected

Table 4. Antioxidant activity, total phenolics, and isoflavones in edible parts of vegetable plants

Common name	*Mean ± SD.*				
	EC_{50} DPPH (μg/mL)	*EC_{50} ABTS (μg/mL)*	*Total phenolics (μg GAE/g)[a]*	*Daidzein (μg/g)[b]*	*Genistein (μg/g)[b]*
Ginger	277.8 ± 10.96	12.41 ± 6.55	12147.8 ± 8552.2	1.41 ± 0.71	0.63 ± 0.28
Plate brush eggplant	501.2 ± 42.06	94.90 ± 5.00	717.4 ± 706.0	6.46 ± 0.70	0.15 ± 0.15
Jackfruit	515.8 ± 7.36	83.44 ± 35.53	804.1 ± 468.6	8.84 ± 1.08	1.9 ± 0.25
Broken bones tree	677.4 ± 24.70	276.2 ± 23.72	191.4 ± 14.66	20.68 ± 1.06	2.72 ± 0.13
Vegetable humming bird	702.4 ± 31.19	73.25 ± 8.30	211.9 ± 45.30	9.7 ± 2.53	1.36 ± 0.48
Chilli pepper	710.4 ± 23.61	91.26 ± 3.16	295.6 ± 38.57	5.91 ± 0.77	1.83 ± 0.20
Cowslip creeper	756.8 ± 57.17	93.86 ± 4.29	245.9 ± 19.22	4.79 ± 1.92	2.49 ± 0.71
Baa-kwaeng-kom*	2243.7 ± 414.0	100.6 ± 12.36	300.9 ± 106.3	ND	ND
Bitter cucumber	2378.1 ± 1658.4	>450	112.0 ± 26.70	9.35 ± 4.33	0.04 ± 0.1
Angled gourd	4390.9 ± 137.9	>1,050	54.73 ± 49.00	0.51 ± 0.41	0.24 ± 0.04
Galangal	>1,000	59.01± 34.09	760.3 ± 675.78	0.55 ± 0.02	0.51 ± 0.01
Banana	>1,225	>1,225	1.43 ± 0.01	18.15 ± 0.67	0.03 ± 0.03
Salaer*	>2,250	>225	579.2 ± 312.59	17.85 ± 11.03	5.95 ± 1.55

* Vegetable name in Thai; [a] expressed in μg GAE/g dry extract; [b] expressed in μg/g dry weight of vegetable; ND = not detected

followed by galangal, vegetable humming bird, jackfruit, and plate brush eggplant, respectively.

The range of total phenolic contents in edible leaf vegetables varied from 21.75 to 14960.9 μg GAE/g dry extract. The highest level was found in Thai copper pod, followed by Ming aralia, Chinese lizard tail, Horapha-chang, and lead tree, respectively. With regard to the total phenolics in edible other-plant parts, the range varied from 1.43 to 12147.8 μg GAE/g dry extract. The highest level was found in ginger, followed by jackfruit, galangal, plate brush eggplant, and Salaer, respectively.

The range of isoflavone levels in edible leaf vegetables varied from 0.53 to 22.53 μg/g dry weights for daidzein, and 0.10 to 20.27 μg/g dry weights for genistein. The highest level of daidzein was found in Thai copper pod, followed by Pak-herd, soap pod, Ming aralia, and Pak-saw, respectively. The highest level of genistein was found in Thai copper pod, followed by rat-tailed radish, Pak-herd, Ming aralia, and Horapha-chang, respectively. With regard to isoflavones in edible other-plant parts, the content varied from 0.51 to 20.68 μg/g dry weights for daidzein, and 0.03 to 5.95 μg/g dry weights for genistein. The highest level of daidzein was found in broken bone tree, followed by banana, Salaer, vegetable humming bird, and bitter cucumber, respectively. The highest level of genistein was found in Salaer, followed by broken bone tree, cowslip creeper, chilli pepper, and vegetable humming bird, respectively.

Comparison of antioxidant activity, total phenolics, and isoflavones between edible leaf and other-plant parts

Antioxidant activity for DPPH assay and total phenolic content of edible leaf vegetables (EC_{50} = 541.2±498.9 μg/mL and 2438.7±3342.7 μg GAE/g dry extract) were significantly higher than those of edible other-plant parts (EC_{50} = 1315.5±1303.4 μg/mL and 1263.3±3281.7 μg GAE/g dry extract). However, antioxidant activity for ABTS assay and isoflavone levels between edible leaf and other-plant parts were not significantly different (Table 5).

Table 5. Comparison of antioxidant activity, total phenolics, and isoflavones contents between edible leaf vegetables and edible parts of vegetable plants

Parameters	*Mean±SD.*		*P value*
	Edible leaf vegetables (n=30)	*Edible plant parts (n=13)*	
EC_{50} DPPH, μg/mL	541.2 ± 498.9	1315.5 ± 1303.4	0.021 *
EC_{50} ABTS, μg/mL	101.5 ± 118.7	98.33 ± 72.03	0.566
Total phenolics, μg GAE /g	2438.7 ± 3342.7	1263.3 ± 3281.7	0.004 **
Daidzein, μg/g	4.30 ± 4.96	8.68±6.96	0.067
Genistein, μg/g	2.23 ± 4.12	1.49 ± 1.71	0.371

*Significantly different at 0.05; **significantly different at 0.01

Table 6. Spearman rank correlation coefficient among antioxidant activity, total phenolics, and isoflavones in vegetables

	Spearman correlation coefficient (r)			
	EC_{50} ABTS	*Total phenolics*	*Daidzein*	*Genistein*
EC_{50} DPPH	0.638**	-0.654**	-0.117	-0.361*
EC_{50} ABTS	-	-0.569**	0.124	-0.092
Total phenolics	-	-	-0.014	0.156
Daidzein	-	-	-	0.377*

*Statistical correlation significance at 0.05, **Statistical correlation significance at 0.01, n=43

Association of antioxidant activity, total phenolics, and isoflavones in vegetables

EC_{50} of DPPH assay was positively associated with EC_{50} of ABTS assay (r = 0.638), and negatively associated with total phenolics and genistein (r = -0.654 and -0,361, respectively). EC_{50} of ABTS assay was negatively associated with total phenolics (r = -0.569). Daidzein levels were positively associated with genistein levels (r = 0.377) (Table 6).

DISCUSSION

The present study found that antioxidant activity and total phenolics of edible leaf vegetables were higher than those of edible other-plant parts. The results are in agreement with the study of Diaz *et al.* (2012), indicating that leaves which are more exposed to sunlight had higher phenolics level. Exposure to a high level of UV radiation increased the levels of phenolics and flavonoids (Corradini *et al.*, 2011; Diaz *et al.*, 2010; Garcia-Marcias *et al.*, 2007). Hence, it could be hypothesised that there are higher levels of antioxidants in edible leaf vegetables than in other-plant parts. Among the 43 vegetables analysed in this study, 10 edible leaf vegetables and 1 other- plant parts exhibited the strongest antioxidants based on a combinative consideration of the results obtained by DPPH, ABTS, and Folin-Ciocalteu method. They were bamboo grass, Pak-wan, Thai copper pod, lead tree, Vietnamese coriander, Chinese lizard tail, Horapha-chang, Ming aralia, kitchen mint, and ginger. The results are in congruence with the study of Chanwitheesuk, Teerawutgulrag & Rakariyatham (2005) who determined total phenolics in some edible plants of Thailand. They found that the 5 vegetable types having high total phenolics were lead tree, Thai copper pod, kitchen mint, Gurma, and Horapha-chang, respectively. The study of Isabelle

et al. (2010) suggests that dark green leaf and brightly-coloured vegetables tend to exhibit high antioxidants. Nonetheless, our results of vegetable types having high antioxidants were very different from the results of other authors. It might be influenced by several factors, including location, climatic conditions, cultivation, harvest, and storage conditions (Deng *et al.*, 2013; Isabelle *et al.*, 2010; Oviasogie *et al., 2009).*

According to the results of antioxidant activities between DPPH and ABTS assays, the activity for DPPH assay was positively associated with the activity for ABTS assay. However, the EC_{50} determined using the ABTS assay was consistently lower than the value determined using the DPPH assay because of the different times of end-point between the assays. ABTS method is often used to confirm the results from DPPH assay because it is based on a similar antioxidant mechanism (Agourram *et al.*, 2013). The study of Floegel *et al.* (2011) suggests that ABTS assay may be more useful than DPPH assay for detecting antioxidant activity in a variety of foods. However, several investigations found that it was difficult to compare the results of both assays (Gramza-Michalowska & Czlapka-Matyasik, 2011). In addition, our results found that the antioxidant activity for DPPH assay was associated with genistein, but not with daidzein. It is generally known that isoflavones, including genistein and daidzeinare are categorised into flavonoid class, which is in phenolic compounds. It is possible that genistein has greater ability to reduce and scavenge for free radicals than daidzein. Several studies indicated that genistein exhibits the most potent antioxidant level due to their number and position in the hydroxyl group (c-4'position) (Arora, Nair & Strasburg, 1998; Rimbach *et al.*, 2003; Sapbamrer, Visavarungroj & Suttajit, 2012).

The interesting finding was that antioxidant activities for DPPH and ABTS assays were associated with total phenolics. The results were in congruence with previous studies, indicating that the samples with high total phenolics exhibited high antioxidant activitiy (Andarwulan *et al.*, 2010; Deng *et al.*, 2013; Diaz *et al.*, 2010; Gramza-Michalowska & Czlapka-Matyasik, 2011; Katsube *et al.*, 2004). One possible explanation is that the chemical structure of total phenolics is responsible for antioxidant activity. Another possibility is that Folin-Ciocalteu assay mechanism is an oxidation/reduction reaction, resulting in its association (Agourram *et al.*, 2013; Katsube *et al.*, 2004; Prior, Wu & Schaich, 2005). However, the correlation found in the present study was not very high (r = -0.654 for DPPH and r = -0.569 for ABTS). It is possible that total phenolic content not only plays an important role in antioxidant activity but also in the interaction of the phenolic compounds with other molecules which might influence the activity (Gramza-Michalowska & Czlapka-Matyasik, 2011). The Folin-Ciocalteu reagent is not specific to phenolic compounds but is often used for phenolic measurement. The reagent can be reduced by many non-phenolic compounds including ascorbic acid, and the reaction occurs through the mechanism of electron transfer. However, many researchers have continued using this measurement for screening total phenolics in the samples (Isabelle *et al.*, 2010).

CONCLUSION

This study concluded that antioxidant activity and total phenolics of edible leaf vegetables were higher than those of edible other-plant parts. Our study found antioxidant activity to be associated with total phenolics due to the structure and the method used to determine total phenolics related to antioxidants. In addition, it is interesting to note that Thai copper pod had the highest levels of phenolic compounds and isoflavones, and strong antioxidant activity. It is suggested that further investigation be carried out on its

properties for antimutagenic, antibacterial, anticarcinogenic, and anti-inflammatory activities.

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Conflict of interest

We declare that we do not have any competing interest.

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