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Phenolic compounds and antioxidant capacities in leaves of fifteen Mao-Luang (*Antidesma thwaitesianum* Müll. Arg.) cultivars

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Abstract The leaf extracts from fifteen Mao-Luang (*Antidesma thwaitesianum*) cultivars found in Northeast Thailand were determined for the phenolic compounds using HPLC analysis and the antioxidant capacities of the leaf extracts were evaluated by ABTS, FRAP and DPPH radical assays. Total phenolic content and total flavonoid contnt were also evaluated. The results revealed that significant ($p < 0.05$) differences in the polyphenolic contents and antioxidants of the studied Mao-Luang cultivars were detected. Quercetin and gallic acid were the main polyphenolics in leaves of all studied Mao-Luang cultivars with the maximum values detected in 'Dongtong No. 2' and 'Thepradit' with the values of 47.21 and 3623.14 mg/100 g DW, respectively. Myricetin, epicatechin and cinnamic acid were detected in the majority of Mao-Luang cultivars. Total phenolic and total flavonoid contents were highest in 'Dongphut No. 2' and 'Huybang' with the values arranged in order of 205.08 mg GAE/100 g DW and 422.12 mg CE/100 g DW. Again, 'Huybang' also showed the maximum antioxidant capacity of 121.03 mmol Fe(II)/g DW (FRAP assay) and 115.86 mmol TE/g DW (ABTS assay). Similarly, 'Wilai No. 2' displayed the highest antioxidant activity of 135.24 mmol VCEAC/g DW (DPPH assay). These findings suggest that polyphenolics and antioxidants in Mao-Luang leaves vary considerably across cultivars.

Keywords: cultivar, local fruit, phytochemical, scavenging

Introduction

Antidesma thwaitesianum Müll. Arg., widely known as Mao-Luang in Thailand, is a wild tropical fruit plant with medicinal value in the family Euphorbiaceae, predominantly distributed in Southeast Asia, particularly in the forest areas of Northeast Thailand (Nuengchamnong and Ingkaninan, 2010; Puangpronpitag *et al*., 2011). The ripen fruits of this plant are consumed fresh or processed into juice or a type of wine (Nuengchamnong and Ingkaninan, 2010). The leaves are edible as fresh vegetables and are believed to be highly nutritious (Dechayont *et al*., 2017). Previous studies have elucidated the potent

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antioxidant activities of Mao-Luang fruits, seeds and marcs, owing to their high quantities of phenolic compounds and flavonoids (Puangpronpitag *et al*., 2008; Hansakul *et al*., 2015). Additionally, the pomace extract of Mao-Luang has been reported to alleviate hypertension and oxidative stress in nitric oxide (NO) deficient rats (Kukongviriyapan *et al*., 2015).

Phenolic acids and flavonoids are bioactive compounds widely distributed in the plant kingdom and could be categorized into various structural groups, such as flavan-3-ols, flavonols, flavones, flavanones, tannins and anthocyanins (Panche *et al*., 2016). It has been documented that flavonols, phenolic acids and anthocyanins act as antioxidant, anti-inflammatory, anticarcinogenic and antibacterial agents, which are beneficial to human health (Puangpronpitag *et al*., 2008; Seeram, 2008; Puangpronpitag *et al*., 2011; Dechayont *et al*., 2012).

Because of its abundance in bioactive compounds, Mao-Luang is extensively cultivated in Northeast Thailand. In particular, Phu Phan National Park, an isolated national park in Northeast Thailand, is renowned for the biodiversity of Mao-Luang cultivars, in which the varieties of Mao-Luang thriving in mountain regions and in the vicinity of Phu Phan Mountains signify a broad genetic diversity (Jorjong *et al*., 2015).

It has been recognized that the profile and content of bioactive compounds in plants are highly dependent on numerous factors, including genotype, climatic conditions and agronomic practices (Skrovankova *et al*., 2015; Auzanneau *et al*., 2018). Mao-Luang fruits of different cultivars harvested from different geographical regions and locations have been reported to contain different amounts of anthocyanins, flavonoids and phenolic acids (Butkhup and Samappito, 2008). Our previously reported study (Jorjong *et al*., 2015) also confirms the influence of cultivar on the quantity of bioactive substances in Mao-Luang fruits. Although many studies have been implemented to examine the bioactive substances and antioxidants in Mao-Luang fruits and seeds, there is limited information on the polyphenolics and antioxidants in leaves of these Mao-Luang cultivars (Hansakul *et al*., 2016; Dechayont *et al*., 2017) and should therefore be examined in order to encourage the use of Mao-Luang leaves for medicinal purposes.

For this purpose, the aim of this study was to examine the polyphenolic content and antioxidants in leaves of fifteen cultivars of Mao-Luang collected from a local orchard in the northeastern region of Thailand. In this attempt, the content of polyphenols was determined by HPLC-DAD analysis and the antioxidant activity was assessed by ABTS, FRAP and DPPH scavenging assays. Total phenolic and total flavonoid contents were also examined.

Materials and methods

Plant materials and sample preparation

Leaves of fifteen Mao-Luang cultivars, namely 'Sangkho No. 2', 'Theppradit', 'Kanlaya', 'Kamma', 'Huybang', 'Wannawong No. 2', 'Wichian', 'Theongway', 'Phusong No. 1', 'Hinkuay', 'Phuangsor', 'Nongbua', 'Wilai No. 2', 'Dongtong No. 2' and 'Dongphut No. 2', were harvested from a local orchard in Northeast Thailand. Third leaves were selected and harvested in the early morning (Oszmiański *et al*., 2009). The leaves were thoroughly cleaned and oven-dried at 65 ℃ for 72 h. After that, the leaves were ground to a fine powder and 0.5 g of the obtained powder was extracted using methanol (10 mL) for 20 min under sonicaton; this extraction method has been proved to be adequate for complete extraction. After 10-min centrifugation at 19,000 ×g, the supernatant was collected for the determination of antioxidant activities and polyphenolic compounds.

Determination of polyphenols by HPLC/DAD analysis

Quantitative analysis of polyphenols was performed on a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) consisting of a model LC-20AD binary solvent pump, a model SIL-10AD autosampler and a model SPD-M20A diode-array detector (DAD). The separation was achieved on an Apollo C_{18} column (250 mm × 4.6 mm i.d., 5 µm, Alltech Associates Inc., Deerfield, IL, USA) protected with an Inertsil ODS-3 guard column (10 mm × 4 mm i.d., 5 µm, GL Science Inc., Tokyo, Japan). The column temperature was set at 40 ℃. Injection volume was 20 µL. Standard polyphenols used in this study included caffeic acid, (+)-catechin, cinnamic acid, chorogenic acid, cyanindin, cyanidin-3-rutinoside, cyanidin-3-o-glucoside, dephinidin, (–)-epicatechin, ferulic acid, gallic acid, kaempferol, luteolin, malvidin 3,5-diglucoside, malvin, myricetin, naringenin, pelargonidin, quercetin, rutin, sinapinic acid, *trans*-resveratrol and vanillic acid.

The mobile phase used for the elution of phenolic acids and flavonoids is composed of solvent A (acetonitrile/water, 2:97.8, v/v) and solvent B (acetonitrile/water, 97.8:2, v/v), both with 0.2% H_3PO_4 with gradient elution. Flavonoids were analyzed at 254 nm using the following gradient program with a flow rate of 0.6 mL/min: 0 min 20% (B), 0–30 min 50% (B), 30–35 min 60% (B), 35–40 min 20% (B), and kept at 20% (B) until the end of the run at 55 min. Linear gradients at 280 nm with a flow rate of 0.8 mL/min were applied for phenolic acids as follows: 0–15 min 91% (B), 15–22 min 81% (B), 22–38 min 82% (B), 38–45 min 77% (B), 70% (B) at 45 min, 45–46 min 20% (B), 46–60

min 95% (B), and kept at 95% (B) until the end of the run at 65 min. The mobile phase for the analysis of anthocyanins was 4% H_3PO_4 in water (A) and acetonitrile (B). The gradient program at a flow rate of 1 mL/min was as follows: 0 min 94% (B), 0–55 min 75% (B), 55–65 min 75% (B), and kept at 75% (B) until the end of the run at 70 min. The analyte was detected at a wavelength of 520 nm.

Polyphenols were identified by comparing retention time and UV absorption spectra with available standards. Quantification was performed with standard curves of external standards generated by plotting HPLC peak areas against the concentrations.

Determination of total phenolic contents

Total phenolic contents (TPC) were determined spectrophotometrically by Folin-Ciocalteau assay (Singleton and Rossi, 1965). Standard solutions of gallic acid at different concentrations (0.5–100 mg/L) were used for the calibration curve. Briefly, 12.5 µL of the leaf extract or gallic acid standard was mixed with 12.5 µL of 10% Folin-Ciocalteu reagent and 125 µL of 20% Na_2CO_3 solution. The mixture was incubated in the dark for 90 min at room temperature and then measured at 760 nm. TPC was expressed as mg gallic acid equivalent per 100 g dry weight (mg GAE/100 g DW).

Determination of total flavonoid contents

Total flavonoid contents (TFC) were quantified using a colorimetric assay (Kim *et al*., 2003) with slight modifications. Standard solutions of catechin at different concentrations (0.5–150 mg/L) were used for the calibration curve. In brief, 25 µL of the leaf extract or catechin standard was mixed with 75 µL of 5% $NaNO_3$ and the mixture was left at room temperature for 5 min. After the addition of 150 µL of 10% $AlCl_3$ and left at room temperature for 5 min, 50 µL of 1 M NaOH was added to the mixture. Then the mixture was determined at 510 nm. TFC was expressed as mg catechin equivalent per 100 g dry weight (mg CE/100 g DW).

DPPH scavenging assay

The antioxidant capacity of the leaf extracts was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) free radical method as described earlier by Akowuah *et al*. (2005) with minor modifications. Standard solutions of vitamin C at different concentrations (0–200 µM) were used for the calibration curve. In brief, 0.2 mM DPPH methanolic solution and the leaf extract were mixed in

equal amounts (100 µL). After thorough mixing and incubation under dark conditions for 1 h, the mixture was determined at 520 nm. The results were expressed in mg vitamin C equivalent antioxidative capacity per g dry weight (mg VCEAC/g DW).

Ferric reducing/antioxidant power assay (FRAP)

The antioxidant capacity of the leaf extracts was determined using FRAP method according to Benzie and Strain (1999) with slight modifications. Standard solutions of iron (II) sulphate at different concentrations (0–500 µM) were used for the calibration curve. FRAP reagent was freshly prepared by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl and 20 mM $FeCl_3$ solution in 1:1:10 (v/v/v) proportions. A 30-µL aliquot of the leaf extract was reacted with 270 µL of FRAP reagent (warmed at 37 ℃ before use) for 30 min and the mixture was determined at 595 nm. The results were expressed as mmol ferrous ion per g dry weight (mmol Fe(II)/g DW).

ABTS scavenging assay

Assessment of the antioxidant capacity of the leaf extracts by ABTS [2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] cation radical scavenging method was conducted as per the method described by Seeram *et al*. (2006) with some modifications. Standard solutions of trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid) at different concentrations (0–500 µM) were used for the calibration curve. ABTS radical cations were prepared combining 2.6 mM potassium persulfate and 7.4 mM ABTS in equal amounts and after thorough mixing the mixture was left to settle for 12 h before use. A 10-µL volume of the leaf extract was mixed with 190 µL of ABTS radical cation solution. After thorough mixing followed by subsequent 2-h incubation, the mixture was measured at 595 nm. The results were expressed in mmol trolox equivalent antioxidative capacity per g dry weight (mmol TEAC/g DW).

Statistical analysis

All the determinations were performed in triplicate and the results are expressed as the mean ± one standard deviation. The data obtained were analysed using one-way analysis of variance and comparison between groups was performed using Duncan's multiple range test. Differences among groups were considered significant at $p < 0.05$.

Results

Polyphenols

The polyphenol profiles of the leaf extracts of fifteen Mao-Luang cultivars are presented in Table 1. It was observed that there were considerable vatiations in the polyphenol contents among Mao-Luang cultivars. Quercetin, myricetin and epicatechin were the major components in Mao-Luang leaves, with quercetin found in all cultivars and the other two compounds detected in almost all cultivars. 'Dongtong No. 2' exhibited the highest content of quercetin (47.21 mg/100 g DW), followed by 'Wannawong No. 2' (27.69 mg/100 g DW) and 'Huybang' (20.57 mg/100 g DW). 'Huybang' also showed the maximum content of epicatechin (9426.37 mg/100 g DW), followed by 'Sangkho No. 2' (5967.87 mg/100 g DW) and 'Theongway' (3791.48 mg/100 DW). Meanwhile, the highest amount of myricetin (5.72 g/100 g DW) was observed in 'Theongway' followed by 'Dongphut No. 2' and 'Sangkho No. 2' with the values of 5.12 and 5.11 mg/100 g DW, respectively. Qercetin-3-o-rutinoside and catechin were found in very few cultivars. Notably, kaempferol, naringenin and trans-resveratrol were not detected in any cultivars. However, 'Huybang' exhibited the highest content of total polyphenols (9451.38 mg/100 g DW) followed by 'Sangkho No. 2' (5980.75 mg/100 g DW) and 'Theongway' (3826.57 mg/100 g DW).

Phenolic acids

The phenolic acid profiles of the leaf extracts of fifteen Mao-Luang cultivars was shown in Table 2. It was found that the leaf extracts of Mao-Luang cultivars showed varying amounts of phenolic acids. Gallic acid was found in all Mao-Luang cultivars while almost all cultivars were observed to contain cinnamic acid. On the other hand, vanillic acid, chlorogenic acid and sinapinic acid were only detected in some cultivars. Meanwhile, caffeic acid and ferulic acid were not found in any cultivars. 'Theppradit' was found to contain the highest content of gallic acid with the value of 3623.14 mg/100 g DW followed by 'Huybang' and 'Sangkhon No. 2' with the values arranged in order of 3172.96 and 2231.72 mg/100 g DW. The greatest amount of cinnamic acid was found in 'Dongtong No. 2' (23.06 mg/100 g DW) followed by 'Wilai No. 2' and 'Nongbua' with the quantities of 16.21 and 15.47 mg/100 g DW, respecitvely. Again, the highest quantity of total phenolic acids were observed in 'Theppradit' with the value of 3641.54 mg/100 g DW followed by 'Huybang' and 'Dongtong No. 2' with the values arranged in order of 3284.42 and 2469.42 mg/100 g DW.

Total phenolics, total flavonoids and antioxidant capacity

The total phenolics, total flavonoids and antioxidant activities of the leaf extracts of fifteen Mao-Luang cultivars are given in Table 3. As presented, it was noted that differences in the total phenolics and total flavonoids were seen among Mao-Luang cultivars. 'Dongphut No. 2' was found to have the highest content of total phenolic compounds with the value of 205.08 mg GAE/100 g DW followed by'Wilai No. 2' and 'Huybang' with the quantities of 203.09 and 200.71 mg/100 g DW, respectively. Meanwhile, the highest amount of total flavonoids was observed in 'Huybang' (422.12 mg CE/100 g DW) followed by 'Dongphut No. 2' (359.89 mg CE/100 g DW) and 'Sangkho No. 2' (338.38 mg CE/100 g DW). 'Hinkuay' and 'Phuangsor' were found to contain the lowest contents of total phenolic compounds (102.78 mg GAE/100 g DW) and total flavonoids (197.06 mg CE/100 g DW), respectively.

The antioxidant activities of the leaf extracts of fifteen Mao-Luang cultivars was evaluated by ABTS, FRAP and DPPH radical assays (Table 3). Again, there were differences in the antioxidant activities of the studied Mao-Luang cultivars. The activities of FRAP varied considerably among Mao-Luang cultivars, with the values ranging from 20.42 to 121.03 mmol Fe(II)/g DW. The maximum activity of FRAP was seen in 'Huybang' with the value of 121.03 mmol Fe(II)/g DW followed by 'Theppradit' (105.01 mmol Fe(II)/g DW) and 'Sangkho No. 2' (95.7 mmol Fe(II)/g DW). The lowest activity of FRAP was observed in 'Hinkuay' with the value of 20.42 mmol Fe(II)/g DW (Table 3).

As indicated by ABTS assay, the antioxidant activitiy among Mao-Luang cultivars ranged from 18.44 to 115.86 mmol TE/g DW. Like to the FRAP value, the maximum ABTS value (115.86 mmol TE/g DW) was observed in 'Huybang', which possessed significantly ($p < 0.05$) stronger antioxidant capacity than other cultivars, followed by 'Theppradit' (91.87 mmol TE/g DW) and 'Sangkho No. 2' (84.09 mmol TE/g DW). Again, the lowest ABTS value of 18.44 mmol TE/g DW was seen in 'Hinkuay' (Table 3).

The antioxidant capacity of the leaf extracts of Mao-Luang cultivars was also assessed by DPPH radical assay and expressed in the VCEAC values. Similar to the FRAP and ABTS values as shown in Table 3, considerable variations in the VCEAC values were detected among Mao-Luang cultivars in a range of 46.29–135.24 mmol VCEAC/g DW. The highest VCEAC value of 135.24 mmol VCEAC/g DW was found in 'Wilai No. 2' followed by 'Sangkho No. 2' and 'Theppradit' with the VCEAC values of 135.07 and 134.18 mmol VCEAC/g DW, respectively. 'Hinkuay' again exhibited the lowest VCEAC value of 46.29 mmol VCEAC/g DW (Table 3).

Table 1. Polyphenol profiles in leaves of fifteen Mao-Luang cultivars

Cultivars	Flavonols					Flavan-3-ols			Flavanone	Stilbene	Total polyphenols
	Quercetin	Quercetin-3-O-rutinoside	Myricetin	Kaempferol	Total flavonols	(+)-Catechin	(-)-Epicatechin	Total flavan-3-ols	Naringenin	*trans*-Resveratrol	
Sangkho No. 2	7.77 ±0.39fg	n.d.	5.11 ±0.18b	n.d.	12.88fgh	n.d.	5967.87 ±20.29b	5967.87 ±20.29b	n.d.	n.d.	5980.75b
Thepradit	15.11 ±1.25d	n.d.	n.d.	n.d.	15.11fg	n.d.	n.d.	n.d.	n.d.	n.d.	15.11j
Kanlaya	9.58 ±0.09ef	72.79 ±4.24a	n.d.	n.d.	82.36a	n.d.	2360.66 ±43.57f	2360.66 ±43.57e	n.d.	n.d.	2443.02de
Kamma	5.33 ±0.13hi	n.d.	3.12 ±0.00e	n.d.	8.45h	n.d.	3636.06 ±25.81d	3636.06 ±25.81c	n.d.	n.d.	3644.51c
Huybang	20.57 ±2.80c	n.d.	4.44 ±0.10cd	n.d.	25.01e	n.d.	9426.373 ±51.87a	9426.373 ±51.87a	n.d.	n.d.	9451.38a
Wannawong No. 2	27.69 ±0.71b	n.d.	4.55 ±0.58bcd	n.d.	31.57d	n.d.	2407.48 ±1.34f	2407.48 ±1.34de	n.d.	n.d.	2439.71de
Wichian	6.72 ±0.44gh	n.d.	4.86 ±0.06bc	n.d.	11.58fgh	n.d.	2625.73 ±94.86e	2625.73 ±94.86d	n.d.	n.d.	2637.97d
Theongway	11.37 ±0.28e	n.d.	5.72 ±0.49a	n.d.	17.09f	n.d.	3791.48 ±49.13c	3791.48 ±49.13c	n.d.	n.d.	3826.57c
Phusong No. 1	6.38 ±0.32ghi	n.d.	3.12 ±0.00e	n.d.	9.50gh	n.d.	1932.78 ±68.57g	1932.78 ±68.57f	n.d.	n.d.	1942.27g
Hinkuay	4.12 ±0.07i	4.60 ±0.18c	3.28 ±0.16e	n.d.	11.99fgh	n.d.	267.75 ±33.81j	267.75 ±33.81h	n.d.	n.d.	433.02i
Phuangsor	5.31 ±1.17hi	n.d.	3.12 ±0.00e	n.d.	8.43h	1416.85 ±33.43	726.567 ±52.42i	1991.150 ±307.77f	n.d.	n.d.	1999.58fg
Nongbua	9.07 ±0.50ef	n.d.	3.12 ±0.00e	n.d.	12.19fgh	n.d.	1243.23 ±27.54h	1243.23 ±27.54g	n.d.	n.d.	1255.42h
Wilai No. 2	10.66 ±0.30e	54.32 ±8.49b	n.d.	n.d.	64.98b	1416.85 ±33.43	742.457 ±24.77i	2169.387 ±10.50ef	n.d.	n.d.	2234.37ef
Dongtong No. 2	47.21 ±1.76a	n.d.	4.15 ±0.04d	n.d.	52.05c	n.d.	n.d.	n.d.	n.d.	n.d.	52.05j
Dongphut No. 2	5.26 ±0.27hi	17.10 ±0.11c	5.12 ±0.14b	n.d.	27.47de	n.d.	2335.97 ±64.28f	2335.967 ±64.28e	n.d.	n.d.	2363.43e
Average	**12.81**	**37.20**	**4.14**	**n.d.**	**26.04**	**1421.89**	**2881.88**	**2688.48**	**n.d.**	**n.d.**	**2714.61**

Results are means ±S.D. of three measurements (in mg/100 mg DW).
n.d. = not detected.
Values followed by different letters in the same column are significantly different ($P < 0.05$).
Total flavonols are the sum of kaempferol, myricetin, qurercetin and quercetin-3-O-rutinoside.
Total flavan-3-ols are the sum of (+)-catechin and (-)-epicatechin
Total polyphenols are the sum of (+)-catechin, (-)-epicatechin, kaempferol, myricetin, naringenin, quercetin-3-O-rutinoside, qurercetin and *trans*-resveratrol.

Table 2. Phenolic acid profiles in leaves of fifteen Mao-Luang cultivars

Cultivars	Hydroxybenzoic acids			Hydroxycinnamic acids						Total phenolic acids
	Gallic acid	Vanillic acid	Total hydroxybenzoic acids	Chlorogenic acid	Caffeic acid	Ferulic acid	Sinapinic acid	Cinnamic acid	Total hydroxycinnamic acids	
Sangkho No. 2	2231.72±118.26c	n.d.	2231.72b	13.79±0.32bc	n.d.	n.d.	n.d.	1.040±0.06g	14.83fg	2246.55abc
Theppradit	3623.14±185.86a	n.d.	3623.14a	15.79±0.48ab	n.d.	n.d.	n.d.	2.617±0.18fg	18.40f	3641.54a
Kanlaya	931.43±63.94e	n.d.	931.43cde	16.69±0.94a	n.d.	n.d.	42.93±1.80cde	2.02±0.05fg	61.64c	993.06abc
Kamma	735.18±39.04e	n.d.	735.18efg	11.20±0.18d	n.d.	n.d.	n.d.	4.097±0.14fg	15.29fg	750.14bc
Huybang	3172.96±288.91b	n.d.	3172.961a	14.63±0.20abc	n.d.	n.d.	n.d.	0.457±0.00g	15.09fg	3284.42ab
Wannawong No. 2	775.44±12.65e	n.d.	775.44ef	n.d.	n.d.	n.d.	48.99±0.11cd	11.63±2.94cd	60.62cd	1519.50abc
Wichian	867.10±104.02e	n.d.	867.10def	n.d.	n.d.	n.d.	39.75±0.08def	6.203±0.91ef	45.95e	856.97bc
Theongway	1352.07±88.36d	n.d.	1352.07c	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1210.95abc
Phusong No. 1	714.34±38.65e	n.d.	714.34efg	n.d.	n.d.	n.d.	n.d.	5.003±0.73fg	5.00g	946.82abc
Hinkuay	231.58±27.94f	n.d.	231.58h	n.d.	n.d.	n.d.	28.08±1.42fg	12.077±1.79bcd	40.15e	443.61c
Phuangsor	450.10±35.89f	7.37±0.59d	457. 47fgh	n.d.	n.d.	n.d.	34.60±4.04efg	11.483±0.71cd	46.08e	405.00c
Nongbua	693.77±0.00e	5.81±0.01d	699.58efg	13.10±0.30cd	n.d.	n.d.	22.15±0.08g	15.467±1.32bc	50.72de	750.30bc
Wilai No. 2	279.36±1.32f	9.99±1.06c	289.34gh	n.d.	n.d.	n.d.	63.23±4.64b	16.213±2.52b	79.45.b	368.79±8.60c
Dongtong No. 2	2290.33±23.80c	52.07±1.09a	2342.39b	n.d.	n.d.	n.d.	103.96±12.18a	23.063±4.08a	127.00a	2469.42abc
Dongphut No. 2	1215.25±44.63d	19.92±0.29b	1235.17cd	n.d.	n.d.	n.d.	53.87±6.61bc	10.093±2.51de	63.96c	1299.13abc
Average	**1304.250**	**19.032**	**1288.37**	**14.20**	**n.d.**	**n.d.**	**48.617**	**8.676**	**42.946**	**1412.41**

Results are means±S.D. of three measurements (in mg/100 mg DW).
n.d. = not detected.
Values followed by different letters in the same column are significantly different ($P < 0.05$).

Table 3. Total phenolic contents, total flavonoid contents and antioxidant activities in leaves of fifteen Mao-Luang cultivars

Cultivars	TPC (mg GAE/100 g DW)	TFC (mg CE/100 g DW)	DPPH (mmol VCEAC/g DW)	FRAP (mmol Fe(II)/g DW)	ABTS (mmol TE/g DW)
Sangkho No. 2	191.08 ±16.48	338.38 ±3.76	135.07 ±0.66	95.7 ±7.74	84.09 ±25.42
Theppradit	192.58 ±3.34	279.42 ±8.6	134.18 ±0.54	105.01 ±9.65	91.87 ±13.32
Kanlaya	182.01 ±16.69	324.90 ±18.33	114.17 ±6.62	45.95 ±9.34	44.37 ±0.72
Kamma	173.91 ±21.09	287.91 ±5.65	95.61 ±5.81	43.83 ±7.23	39.10 ±6.89
Huybang	200.71 ±7.19	422.12 ±10.19	117.74 ±1.7	121.03 ±16.33	115.86 ±9.99
Wannawong No. 2	195.25 ±13.43	219.25 ±6.08	104.53 ±6.72	43.47 ±1.9	36.98 ±0.68
Wichian	187.27 ±7.01	199.98 ±22.49	107.72 ±10.68	50.99 ±0.81	44.7 ±3.01
Theongway	189.43 ±1.51	197.34 ±15.67	116.16 ±12.38	70.23 ±8.49	63.32 ±6.23
Phusong No. 1	179.75 ±12.32	211.32 ±7.56	73.03 ±14.67	49.92 ±9.73	41.2 ±1.81
Hinkuay	102.78 ±19.79	234.72 ±54.16	46.29 ±21.43	20.42 ±3.19	18.44 ±3.93
Phuangsor	171.11 ±8.89	197.06 ±37.47	94.51 ±7.8	42.22 ±17.07	36.25 ±6.46
Nongbua	172.49 ±16.55	259.26 ±7.82	107.97 ±21.12	38.15 ±2.9	45.06 ±2.09
Wilai No. 2	203.09 ±3.18	301.45 ±25.24	135.24 ±6.162	54.59 ±6.13	61.18 ±5.47
Dongtong No. 2	200.00 ±8.31	320.7 ±20.39	131.42 ±2.52	82.52 ±1.21	70.61 ±3.75
Dongphut No. 2	205.08 ±5.46	359.89 ±31.57	127.61 ±6.44	59.5 ±3.56	49.86 ±0.76
Average	**183.1**	**272.52**	**109.42**	**61.57**	**56.19**

Results are means ± SD of three measurements.

Discussion

Determination of polyphenols in the leaf extracts of fifteen Mao-Luang cultivars was carried out using HPLC/DAD analysis. In general, the same polyphenols were detected in all studied cultivars with differences in their relative levels. However, some types of polyphenols were detected in certain cultivars. The most abundant polyphenol was epicatechin (flavan-3-ols) which ranged from 267.75 mg/100 g DW in 'Hinkuay' to 9426.37 mg/100 g DW in 'Huybang'. The beneficial effects of polyphenols on human health, the leaf extracts of Mao-Luang cultivars evaluated in this study were more benficial than other fruits and also other plant parts partly because of the greater contents of flavan-3-ols. The amount of falvan-3-ols in leaf extracts of Mao-Luang cultivars in the present study was much greater than that in our previous study (Jorjong *et al*., 2015) which reported the total content of flavan-3-ols in a range of 292.53 –1445.89 mg/100 g DW in Mao-Luang fruits. Moreover, the whole berry of Ekşikara grape (*Vitis vinifera* L.) grown at high altitude was reported to have lower contents of major flavan-3-ols (21.18–26.27 mg/100 g DW for catechin and 3.98–37.47 mg/100 g DW for epicatechin) (Coklar, 2017). Lowbush blueberries have also been reported to contain the lower amounts of flavan-3-ols (epicatechin) ranging from 2 mg/100 g DW to 80 mg/100 g DW (Gibson *et al*., 2013). Additionally, epicatechin in wild blueberry, raspberry, strawberry and sour cherry has been reported to range from 1.36 mg/100 g DW to 9.58 mg/100 g DW (Levaj *et al*., 2010).

It has been reported that rutin (quercetin-3-O-rutinoside), quercetin and myricetin display superior antioxidant activities than traditional vitamins (Miean and Mohamed, 2001). In the present study, quercetin and myricetin were the major flavonols in fifteen Mao-Luang cultivars. The quantity of quercetin varied from 4.12 mg/100 g DW in 'Hinkuay' to 47.21 mg/100 g DW in 'Dongtong No. 2' while the content of myricetin range from 3.12 mg/100 g DW in 'Kamma', 'Phusong No. 1', 'Phuangsor', and 'Nongbua', to 5.72 mg/100 g DW in 'Theongway'. The amount of quercetin in Mao-Luang cultivars studied was greater than that in two strawberry cultivars ('Korona' 1.4–5.5 mg/100 g DW and 'Tufts' 5.9–11.2 mg/100 g DW) while the quantity of myricetin was comparable (Mahmood *et al*., 2012).

Phenolic acids, being secondary plant metabolites ubiquitous in plant foods, represent one of the most pivotal phytochemicals with their quantities defining the flavor characteristics and quality of fruits (Oszmiański *et al*., 2009). In the present study, cinnamic acid and gallic acid were the major phenolic acids in the leaf extracts of studied Mao-Luang cultivars. The amounts of phenolic acids in fruits and other plant parts are influenced by cultivar, preharvest and postharvest conditions, stage of at-harvest maturity and

processing (Mattila *et al*., 2006). Gallic acid is among other phenolic acids highly absorbed into the human body (Manach *et al*., 2005) and exhibits a positive effect against tumor cells (Pellegrina *et al*., 2005).

Total phenolic content of Mao-Luang cultivars was assessed based on the Folin–Cioacalteu method, which relies on the reducing power of polyphenolic hydroxyl groups and the different reactions of different polyphenols to the Folin–Cioacalteu's reagent (Lin and Tang, 2007). This assay is fast and extensively employed. In the present study, the total phenolic content in the leaf extracts of Mao-Luang cultivars was in a range of 102.78–205.08 mg GAE/100 g DW with the mean value of 183.1 mg GAE/100 g DW (Table 3), which was slightly lower than that reported for black mulberry (183.6–248.3 mg GAE/100 g DW) (Ercisli *et al*., 2010).

It has been elucidated that flavonoids possess a variety of pharmacological functions, such as anti-aging, anti-allergic, antioxidant, anticarcinogenic, antiallergenic, antiviral and anti-inflammatory effects (Miean and Mohamed, 2001; Orak, 2007). In the present study, the total flavonoid content in the leaf extracts of Mao-Luang cultivars varied from 197.06 to 422.12 mg CE/100 g DW (Table 3), which was great than that presented in ripe cherry fruits (51.80 mg CE/100 g DW) (Mahmood *et al*., 2013) and in gooseberries (42 mg CE/100 g DW) (Namiesnik *et al*., 2013).

Antioxidants are reckoned as one of the important molecules that have the potential to protect organisms from oxidative damage resulted from free radicals (Manach *et al*., 2005; Orak, 2007). In general, polyphenolic compounds in plants are related to the reducing power, donating a hydrogen atom to break the free-radical chain (Orak, 2007). In the present study, the antioxidant capacity of Mao-Luang cultivars was evaluated based on the potential to reduce Fe^{3+} to Fe^{2+} in FRAP reagent. Apart from that ABTS radical cation assay was also employed for determining the free radical-scavenging capacities of Mao-Luang cultivars. As shown in Table 3, the ABTS values (18.44–115.86 mmol TE/g DW) in the present study were greater than those reported for strawberry, blackberry and blueberry, which ranged from 0.04 mmol TE/g DW to 0.14 mmol TE/g DW (Huang *et al*., 2012). Also, the ABTS values in Mao-Luang cultivars in the present study were greater than those presented in blueberry (*Vaccinium* sp.) varieties planted in Brazil (1.24–2.44 mmol TE/g) (Rodrigues *et al*., 2011). DPPH radical assay was also used to assess the capability of the leaf extracts of Mao-Luang cultives to scavenge the DPPH radical. Choosing the most suitable assay to evaluate antioxidant capacity is not always a simple task. While the ABTS and FRAP assays are in general appropriate for hydrophilic molecules, the DPPH assay can be used

repeatedly with organic/aqueous samples possessing lipophilic and hydrophilic substances (Namiesnik *et al*., 2013).

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