ฤทธิ์ต้านออกซิเดชันที่สัมพันธ์กับสารฟีนอลิกและสารฟลาโวนอยด์จากเมล็ดของโคลงเคลงยวน

Antioxidant Activity Related to Phenolic and Flavonoid Compounds from Seeds of *Melastoma saigonense*

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บทคัดย่อ

งานวิจัยนี้ทำการหาปริมาณฟีนอลิกรวม ปริมาณฟลาโวนอยด์รวม ฤทธิ์ต้านออกซิเดชันรวมถึงระบุชนิดสารฟีนอลิกและฟลาโวนอยด์หลักจากส่วนสกัดเมล็ดสดของโคลงเคลงยวน (*Melastoma saigonense* (Kuntze) Merr.) โดยนำเมล็ดสดแช่ในตัวทำละลายเมทานอล จากนั้นนำมาสกัดแยกส่วนตามความมีขั้วของตัวทำละลายได้ส่วนสกัดทั้งหมด 4 ส่วน ได้แก่ *เอ็น*-เฮกเซน เอทิล-อะซิเตท *เอ็น*-บิวทานอล และน้ำ ตามลำดับ ผลการศึกษาพบว่าส่วนสกัดเอทิลอะซิเตทมีปริมาณฟีนอลิกรวม (967.22 ± 38.13 mg GAE/g DE) และปริมาณฟลาโวนอยด์รวม (850.84 ± 14.42 mg GAE/g DE) มากที่สุด และยังแสดงฤทธิ์ต้านออกซิเดชันสูงที่สุดเมื่อทดสอบด้วยวิธี DPPH (IC_{50} = 35.91 ± 0.16 μg/mL) ABTS (IC_{50} = 21.14 ± 0.56 μg/mL) และ FRAP (30.20 ± 0.52 mmol Fe(II)/g DE) ในการทดลองยังพบว่าปริมาณฟีนอลิกรวมและปริมาณฟลาโวนอยด์รวมมีความสัมพันธ์เชิงบวกกับฤทธิ์ต้านออกซิเดชัน เมื่อทำการระบุชนิดสารฟีนอลิกและฟลาโวนอยด์ในส่วนสกัดเอทิลอะซิเตทด้วย RP-HPLC พบว่ามีปริมาณกรดซิแนพพิคและไมริซิทินมากที่สุด ตามลำดับ ดังนั้นสารสกัดจากเมล็ดโคลงเคลงยวนจึงอาจเป็นตัวเลือกที่ดีสำหรับนำมาใช้เป็นผลิตภัณฑ์อาหารเสริมและผลิตภัณฑ์สมุนไพรธรรมชาติ

ABSTRACT

This research focuses on the assessment of total phenolic and flavonoid contents, antioxidant activity, as well as phenolics and flavonoids characterization of crude extracts from seeds of *Melastoma saigonense* (Kuntze) Merr.Fresh seeds of *M. saigonense* were extracted successively with methanol, followed by sequential fractionation based on solvent polarity to afford four crude extracts, including *n*-hexane, ethyl acetate, *n*-butanol and aqueous extracts, respectively. The results revealed that the ethyl acetate extract showed the highest total

phenolic (967.22 ± 38.13 mg GAE/g DE) and flavonoid (850.84 ± 14.42 mg GAE/g DE) contents. Moreover, it also showed the highest antioxidant activity when determined by the DPPH (IC_{50} = 35.91 ± 0.16 μg/mL), ABTS (IC_{50} = 21.14 ± 0.56 μg/mL) and FRAP (30.20 ± 0.52 mmol Fe(II)/g DE) methods. The total phenolic and flavonoid contents of the extracts were positively correlated with the antioxidant activity. RP-HPLC analysis suggested that sinapic acid is the predominant phenolic acid found in the ethyl acetate extract, while the predominant flavonoid is myricetin. Thus, the seed extracts of *M. saigonense* might be considered as a good candidate to use as a dietary supplement and natural medicinal products.

คำสำคัญ: โคลงเคลงยวน ปริมาณฟีนอลิกรวม ปริมาณฟลาโวนอยด์รวม ฤทธิ์ต้านออกซิเดชัน

Keywords: *Melastoma saigonense*, Total phenolic content, Total flavonoid content, Antioxidant activity

INTRODUCTION

Reactive oxygen species (ROS) generated in the human body play critical biological roles such as cellular signaling and homeostasis (Prasad et al., 2017). Overproduction of ROS, however, results in oxidative stress, which can cause damage to cell structures, leading to aging and various diseases (Arana et al., 2012). An evaluation of the antioxidant activity of edible plants is of interest since natural compounds that are found to be safe can be therapeutic with fewer adverse side effects than synthetic medicines (Chiasson et al., 2002). Several traditional medicinal plants are excellent sources of phenolics and flavonoids which have been reported to possess diverse pharmacological capacities including antioxidant (Chang et al., 2016), antidiabetic (Irondi et al., 2015), anticancer (Dai and Mumper, 2010) and anti-inflammatory (Benayad et al., 2014) activities.

Melastomataceae is a family of dicotyledonnous flowering plants comprising more than 4,000 species. *Melastoma malabathricum*, one of eight species found in Thailand, has been demonstrated to possess various pharmacological effects such as antihyperlipidaemic and anti-diabetic (Balamurugan et al., 2014), anticancer (Roslen et al., 2014) and hepatoprotective activities (Kamisan et al., 2013). *Melastoma saigonense* (Kuntze) Merr. is an edible plant, commonly known in Thailand as 'Khlong Khleng Yuan'. Traditional folk medicine has used the roots of *M. saigonense* for relieving fever, enhancing appetite, and improving the performance of the kidney and liver. The fruits of *M. saigonense* are capsules, and the dry dehiscent fruit reveals aril-covered seeds that can be eaten and taste similar to mulberry fruit. To our knowledge, antioxidation and phytochemicals of *M. saigonense* seed have not yet been reported. Thus, the present study aimed to evaluate the antioxidant capacities of seed extracts from *M. saigonense* and characterize their phenolic and flavonoid contents. This information might be useful to consumers who seek scientific justification for the medicinal use of the plant.

RESEARCH METHODOLOGY

Plant materials

M. saigonense grown in Sakon Nakhon province, Thailand, was used as the source for plant samples. The dehiscent fruits of *M. saigonense* were collected, from which the aril-covered seeds were removed and used for extraction.

Preparation of seed extracts

The fresh seeds (500 g) of *M. saigonense* were ground using an electric blender and then soaked with methanol (1:10 w/v) at room temperature for 72 h.

After filtration, the extracted solution was collected, and the seeds were re-extracted twice using the same procedure. All the extracts were pooled and concentrated under reduced pressure using a rotary evaporator maintained at 40 °C. The concentrated extract was suspended in distilled water and sequentially partitioned with *n*-hexane, ethyl acetate, and *n*-butanol (1:1 v/v). All the partitioned extracts were further concentrated to give crude *n*-hexane (HE, 1.02 g), ethyl acetate (EA, 42.73 g), *n*-butanol (BU, 18.18 g) and residual aqueous (AQ, 86.82 g) extracts, which were then stored at 4 °C until used.

Determination of total phenolic content

The total phenolic content (TPC) was determined using Folin-Ciocalteu reagent, and gallic acid was used as the phenolic standard, according to the method of Sulaiman et al. (2011) with some modifications. The sample (170 μL) was mixed with 1.0 mL of distilled water and 83 μL of Folin-Ciocalteu reagent, and the mixture was allowed to stand for 5 min. Then, 250 μL of sodium carbonate (75 g/L) was added and the mixture was left to react for 2 h at room temperature. The absorbance of the resulting solution was measured at 760 nm using a UV-VIS spectrophotometer (Lambda 35 UV-VIS spectro photometer, PerkinElmer Inc., Waltham, USA). The TPC was expressed as mg gallic acid equivalents per gram of dried extract (mg GAE/g DE).

Determination of total flavonoid content

The total flavonoid content (TFC) was evaluated according to the method described previously (Lin et al., 2010), using quercetin as the flavonoid standard. The sample (500 μL) was mixed with 100 μL of 50 g/L sodium nitrite solution and the mixture was allowed to stand for 6 min before 200 μL of 100 g/L aluminium chloride solution was added. After incubation for 5 min, the mixture was added with 500 μL of 1 mol/L sodium hydroxide and the total volume was made up to 1.5 mL with distilled water. The absorbance of the reaction mixture was measured at 510 nm using the UV-VIS spectrophotometer. The TFC was expressed as mg quercetin equivalents per gram of dried extract (mg QE/g DE).

Determination of DPPH radical scavenging activity

The DPPH radical scavenging was performed using the method modified from Hussain et al. (2013). A 0.5 mL volume of the sample was added to 1 mL of freshly prepared 0.1 mmol/L DPPH in methanol solution and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm by the UV-VIS spectrophotometer. Trolox and ascorbic acid were used as positive controls, while methanol mixed with 0.1 mmol/L DPPH solution was used as a blank control. The DPPH radical scavenging activity, or DPPH inhibition, was calculated as follows:

$$\text{DPPH inhibition (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where A_{sample} and $A_{control}$ are the absorbance of the sample and the blank control, respectively. The antioxidant activity was expressed as the IC_{50} value, which is the concentration of the sample that is required for 50% inhibition.

Determination of ABTS radical scavenging activity

The measurements of ABTS radical scavenging activity were performed according to the method described previously (Daniel et al., 2011). A 7 mmol/L ABTS solution was mixed with 2.45 mmol/L potassium persulfate solution at the ratio of 1:1 (v/v) to generate $ABTS^{\bullet+}$ and allowed to react for 24 h at room temperature in the dark. The absorbance value of the $ABTS^{\bullet+}$solution was adjusted by adding distilled water to get 0.712±0.006 at 734 nm. A 500 μL volume of the sample was added with 500 μL of the $ABTS^{\bullet+}$ solution

and incubated at room temperature in the dark for 30 min before measuring the absorbance at 734 nm using the UV-VIS spectrophotometer. The ABTS radical scavenging ability was calculated as follows:

$$\text{ABTS inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where A_{sample} and $A_{control}$ are the absorbance of the sample and the blank control, respectively. The antioxidant activity was expressed as the IC_{50} value.

Determination of ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) was determined according to the method described by Zhang et al. (2013). The FRAP reagent was prepared by mixing 50 mL of 300 mmol/L acetate buffer pH 3.6 with 5 mL of 10 mmol/L TPTZ in 40 mmol/L of HCl and 5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ solution and incubating the mixture at 37 °C before being used. Then, 100 μL of the sample was added to 1.4 mL of the FRAP reagent, and the mixture was incubated for 30 min at room temperature in the dark. The absorbance of the reaction mixture was measured at 593 nm using the UV-VIS spectrophotometer. The antioxidant power was expressed as mmol Fe(II) per gram of dried extract (mmol Fe(II)/g DE).

Analysis of phenolics and flavonoids using reversed-phase HPLC

Identification and quantification of phenolic acids and flavonoids were conducted by reversed-phase HPLC (RP-HPLC) using LC-20AC pumps with SPD-M20A photodiode array detector (Shimadzu Corp, Kyoto, Japan). The chromatographic separations were carried out on a C-18 Inertsil ODS-3 column (4.6 mm x 250 mm, 5 μm; Hichrom Limited, Berkshire, UK) according to the method described previously (Butsat and Siriamornpun, 2010). The mobile phases were solvent A (acetic acid, pH 2.74) and solvent B (acetonitrile) which were used at flow rates of 0.8 mL/min. The gradient elution was performed according to the following sequence: from 0 to 5 min, a linear gradient of solvent B from 5 to 9%; from 5 to 15 min, 9% of solvent B; from 15 to 22 min, a linear gradient of solvent B from 9 to 11%; from 22 to 38 min, a linear gradient of solvent B from 11 to 18%, from 38 to 43 min, a linear gradient of solvent B from 18 to 23%; from 43 to 44 min, a linear gradient of solvent B from 23 to 90%; from 44 to 45 min, a linear gradient of solvent B from 90 to 80%; from 45 to 55 min, isocratic solvent B at 80%; from 55 to 60 min, a linear gradient of solvent B from 80 to 5%. A re-equilibration period of 5 min with solvent B held at 5% was used between individual runs. The operating conditions were as follows: the column temperature was 38 °C, the injection volume was 20 μL, and the UV-photodiode array detector was tuned to 280 nm for the analysis of phenolic acids or 370 nm for the analysis of flavonoids. Phenolic compounds were identified by comparing their retention times with those of the external standard compounds.

Statistical analysis

Each experiment was conducted in triplicate. Data were analyzed using the SPSS statistical software for Windows. Analysis of variance was used to test any differences in means. Duncan’s multiple range test was used to determine significant differences. Correlations among different assays were calculated using Pearson’s correlation coefficient (*r*).

RESULTS AND DISCUSSION

Total phenolics and total flavonoid contents

The methanol extract from seeds of *M. saigonense* was partitioned sequentially using solvents with increasing polarity to give HE, EA, BU, and AQ extracts, respectively. TPC and TFC of the crude extracts present in Table 1. The AQ extract had the highest yield by mass, followed by the EA, BU, and HE extracts, in descending order. As different phenolic compounds could have different responses to the Folin-ciocalteu reagent, the TPC was expressed as mg GAE/g DE, which corresponded to the mean response of all the major phenolic compounds in the samples. Likewise, the TFC was expressed as mg QE/g DE.

Table 1 reveals that the TPC and TFC of the extracts were significantly different ($p<0.05$). The TPC ranged from 233.46 to 967.22 mg GAE/g DE, while the TFC was undetectable in the HE, but it ranged from 359.96 to 850.84 mg QE/g DE in other extracts. The EA contained the highest TPC (967.22 mg GAE/g DE) and TFC (850.84 mg QE/g DE), followed by the BU, AQ, and HE. The phenolic compounds were found predominantly in moderate to high polarity portions. The higher TPC than the TFC in the corresponding extracts suggests that the extracts had non-flavonoid phenolic substances. Nevertheless, the present results reveal that the seeds of *M. saigonense* are an abundant source of phenolic compounds and are therefore rich in natural antioxidants and likely to possess other bioactivities, such as anti-inflammation, anti-atherosclerosis, anti-carcinogenesis, and anti-diabetes (Krishnasamy and Muthusamy, 2015).

Table 1 Total phenolic and total flavonoid contents in seed extracts of *M. saigonense*

Seed Extracts	TPC (mg GAE/g DE)	TFC (mg QE/g DE)
HE	233.46 ± 5.91^{d}	nd
EA	967.22 ± 38.13^{a}	850.84 ± 14.42^{a}
BU	793.60 ± 36.50^{b}	794.09 ± 5.97^{b}
AQ	520.77 ± 19.30^{c}	359.96 ± 10.24^{c}

Data of TPC and TFC are presented as mean ± SD (n=3). Different letters (a, b, c, d) in the same column represent significant differences at $p<0.05$. TPC, total phenolic content; TFC, total flavonoid content, HE, hexane extract; EA, ethyl acetate extract; BU, butanol extract; AQ, aqueous extract; GAE, gallic acid equivalents; QE, quercetin equivalents; DE, dried extract; nd, not detected

Antioxidant activities

Measurements of antioxidant activity using various methods with different reaction mechanisms are necessary in order to ensure reliable results. In this study, the FRAP and the ABTS and DPPH free radicals scavenging capacities of the seed extracts were measured, compared with those of the known antioxidants, trolox, and ascorbic acid (Table 2).

The DPPH assay is widely used for antioxidant studies due to its simplicity and sensitivity. $DPPH^{\bullet}$ is a stable radical with strong absorption at 517 nm. When DPPH radical becomes neutralized, the absorption at 517 nm decreases proportionately with the loss of the DPPH radical (Petlevski et al., 2013). In the assay, the antioxidant donates hydrogen to the diphenylpicryl-hydrazine group, resulting in the loss of the DPPH radical (AK and Gülçin, 2008), and thereby a lower value of IC_{50} indicates a higher antioxidant power. Table 2 reveals that there were significant differences among the IC_{50} values of all the sample extracts, while the IC_{50} of the EA was not significantly different from those of the well-known antioxidants, trolox and ascorbic acid ($p<0.05$). Among the seed extracts, the EA

exhibited the highest DPPH radical scavenging activity (IC_{50} 35.91 μg/mL).

The principles of the ABTS and the DPPH methods are similar. In the ABTS assay, ABTS is converted to its stable radical cation whose absorption at 734 nm is decreased when reacting with antioxidants (Abirami et al., 2014). The results of the ABTS radical scavenging activity in Table 2 shows that all the seed extracts had significantly different IC_{50} ($p<0.05$), with the values varied from 21.14 to 486.56 μg/mL. The order of the ABTS radical scavenging activity was EA>BU>AQ>HE, which is in accordance with the results of the DPPH free radical scavenging activity. In the ABTS assay, the IC_{50} values of trolox and ascorbic acid were slightly lower than that of the EA extract.

The same order of the phenolic contents in Table 1and the radicals scavenging activities in Table 2 (EA>BU>AQ>HE) clearly suggests that the seed extracts which contained higher phenolic and flavonoid contents were better scavengers of the DPPH and ABTS radicals. These results correspond with previous studies which reported that flavonoids and phenolic acids had been shown to have the ability to quench the free radicals (Ebrahimzadeh et al., 2010).

Table 2 Antioxidant activities of *M. saigonense* seed extracts

Seed extracts	$DPPH^{\bullet}$ IC_{50}(μg/mL)	$ABTS^{\bullet+}$ IC_{50}(μg/mL)	FRAP (mmol Fe(II)/g DE)
HE	928.05 ± 39.09^{a}	486.56 ± 4.38^{a}	4.97 ± 0.04^{e}
EA	35.91 ± 0.16^{d}	21.14 ± 0.56^{d}	30.20 ± 0.52^{c}
BU	88.77 ± 0.84^{c}	44.54 ± 0.62^{c}	11.35 ± 0.12^{d}
AQ	273.82 ± 2.55^{b}	84.50 ± 1.22^{b}	4.85 ± 0.05^{e}
Trolox*	10.55 ± 0.02^{d}	7.27 ± 0.13^{e}	129.00 ± 1.20^{b}
Ascorbic acid*	7.96 ± 0.09^{d}	4.86 ± 0.05^{e}	227.80 ± 7.16^{a}

Data are presented as mean ± SD (n=3). Different letters (a, b, c, d, e) in the same column represent significant differences at $p<0.05$. DE, dried extract; *Standard antioxidants

The principle of the FRAP assay relies on the ability of the antioxidants to reduce the TPTZ-Fe(III) complex to the blue-colored TPTZ-Fe(II) complex and thus the FRAP values reflect the ferric reducing power. Table 2 reveals that the FRAP and the DPPH and ABTS free radicals scavenging results had the same trend, in which the EA and BU extracts showed higher antioxidant power than the AQ and HE extracts, suggesting that the structures of the major antioxidants in the *M. saigonense* seed extracts were relatively medium polar. The FRAP values range of the extracts were in the order: ascorbic acid>trolox>EA>BU>HE, AQ ($p<0.05$). Among the seed extracts, EA extract had the highest reducing power, while HE and AQ extracts had the lowest reducing power. The slightly different patterns of the antioxidant activities determined by the DPPH, ABTS, and FRAP assays were probably attributed to the different reaction mechanisms of the methods.

Correlation analysis

Table 3 shows the correlation coefficients (r) of the TPC, TFC, and the antioxidant activity of the seed extracts of *M. saigonense* using Pearson test. The correlation between the TPC and TFC (r = 0.953 at $p<0.01$) indicates that flavonoids were the predominant phenolic compounds in the seed extracts. In addition, the results suggested that the TPC, TFC, antioxidant activity (DPPH, ABTS, and FRAP

assays) were strongly positively correlated, with the *r*-values ranged from 0.770 to 0.993 ($p<0.05$). These results confirmed that phenolic compounds, including flavonoids, were the key contributors to the antioxidant activity, which are in agreement with previous studies (Hsieh et al., 2010).

Phenolics and flavonoids characterization

Several authentic phenolic acids and flavonoids were used as the standard for RP-HPLC analysis to characterize phenolics and flavonoids in crude EA, BU, and AQ extracts from seeds of *M. saigonense* (Table 4 and Figure 1). The results showed that the amounts of each phenolic acids in each extract were significantly different ($p<0.05$). All the phenolic acids examined were present in all the extracts, while rutin, myricetin, quercetin, and kaempferol were detected in the EA and BU extracts, but only myricetin, quercetin, and kaempferol were detected in the AQ extract. The results also showed that the EA extract contained the highest contents of the total phenolic acids and total flavonoids investigated, followed by the BU and AQ extracts. The flavonoids, myricetin, and quercetin were the predominant phenolics detected in all the seed extracts. Sinapic acid,gallic acid and ferulic acid were found to be the major phenolic acids in both the EA and BU extracts. These phenolic acids were likely to have contributed to the highest antioxidant activities of the EA due to the presence of hydroxyl and methoxy groups in their structures, and the methoxy group is in the ortho position to the hydroxyl group increased the antioxidant activity (Natella et al., 1999; Eom et al., 2012). Also, the presence of CH=CH-COOH group in the structures of sinapic acid and ferulic acid could increase the antioxidant activity (Muchuweti et al., 2007).

Table 3 Pearson's correlation coefficients of TPC, TFC, and selected antioxidant activities

	TPC	TFC	$DPPH^{\bullet}$	$ABTS^{\bullet+}$	FRAP
TPC	1	0.953^{**}	-0.930^{**}	-0.879^{**}	0.831^{**}
TFC	-	1	-0.993^{**}	-0.962^{**}	0.770^{*}
$DPPH^{\bullet}$	-	-	1	0.991^{**}	-0.488^{*}
$ABTS^{\bullet+}$	-	-	-	1	-0.444
FRAP	-	-	-	-	1

*Significant correlation at $p<0.05$; **Significant correlation at $p<0.01$; TPC, total phenolic content; TFC, total flavonoid content; $DPPH^{\bullet}$, antioxidant activity (IC_{50}) based on DPPH assay; $ABTS^{\bullet+}$, antioxidant activity (IC_{50}) based on ABTS assay; FRAP, ferric reducing antioxidant power

Flavonoids such as myricetin and quercetin were shown to be predominant in all the extracts, but their highest amounts were detected in the EA extract (Table 4). Myricetin exhibits strong antioxidant, anticancer, antidiabetic and anti-inflammatory activities, due to the C_2-C_3 double bond, the OH group, and the catechol in the B-ring (Semwal et al., 2016). Quercetin possesses strong antioxidant, antiviral, antibacterial, anticarcinogenic, and anti-inflammatory activities, due to the presence of the 3-OH and 4-OH groups in the B-ring (Shahidi and Ambigaipalan, 2015). Thus, the highest antioxidant activity of the EA extract might result from the highest contents of myricetin and quercetin.

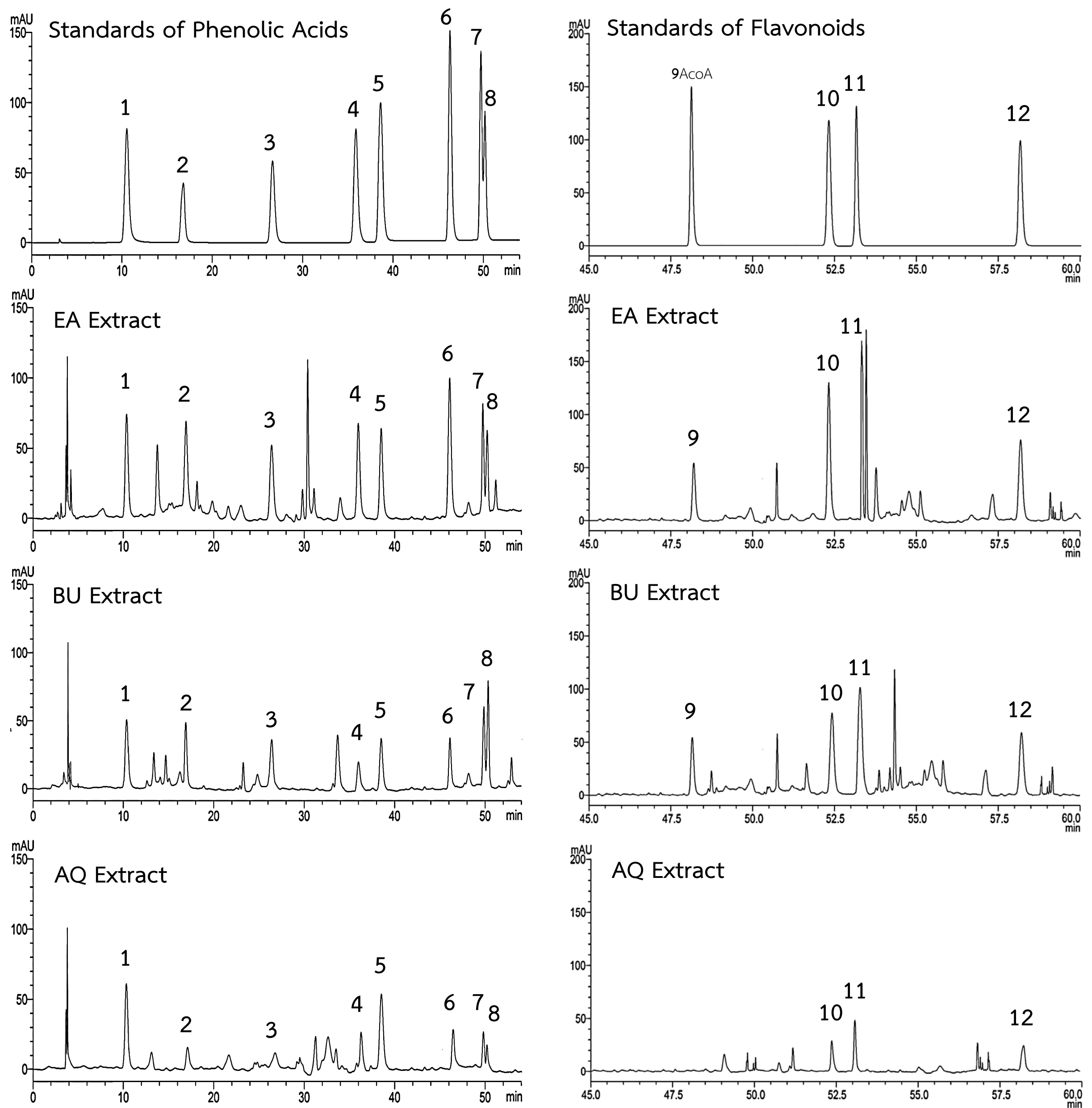

Figure 1. RP-HPLC chromatograms of the seed extracts of *M. saigonense* compared with the standards of phenolic acids including Gallic acid (**1**), Protocatechuic acid (**2**), Hydroxybenzoic acid (**3**), Caffeic acid (**4**), Syringic acid (**5**), *p*-Coumaric acid (**6**), Ferulic acid (**7**), and Sinapic acid (**8**) and the standards of flavonoids including Rutin (**9**), Myricetin (**10**), Quercetin (**11**), and Kaempferol (**12**).

Table 4 Phenolic acids and flavonoids in the seed extracts of *M. saigonense* (µg/g DE)

	Seed Extracts		
	EA	BU	AQ
Phenolic acids			
Gallic acid	493.85±0.84[a]	265.31±0.69[b]	164.83±0.29[c]
Protocatechuic acid	84.01±1.34[a]	65.90±0.41[b]	31.87±0.77[c]
p-Hydroxybenzoic acid	78.19±0.05[a]	65.62±0.86[b]	35.69±0.86[c]
Syringic acid	235.12±1.77[a]	115.09±2.67[b]	110.64±1.45[c]
Caffeic acid	123.89±2.12 [a]	87.22±1.02[b]	52.09±0.69[c]
p-Coumaric acid	129.12±0.06[b]	135.45±2.01[a]	43.21±0.55[c]
Ferulic acid	477.47±0.18[a]	409.21±1.64[b]	95.32±0.69[c]
Sinapic acid	668.35±0.51[a]	485.54±3.34[b]	71.02±0.11[c]
Flavonoids			
Rutin	252.10±0.47[a]	232.32±0.86[b]	nd
Myricetin	2,308.06±4.96[a]	875.69±0.86[b]	256.69±0.33[c]
Quercetin	2,162.65±6.83[a]	1,035.34±3.86[b]	314.73±2.97[c]
Kaempferol	393.15±0.41[a]	336.29±1.46[b]	143.82±0.05[c]
Total	7,405.96±19.54[a]	4,108.98±19.68[b]	1,319.91±8.76[c]

Data are presented as mean ± SD (n=3). Different letters (a, b, c) in the same row represent significant differences at $p<0.05$. DE, dried extract; nd, not detected

CONCLUSIONS

In the present study, the phenolic contents, as well as antioxidant activity of various *M. saigonense* seed extracts, were analyzed. The EA extract was shown to possess the highest contents of phenolic compounds and antioxidant activity when determined by either the radicals scavenging or the reducing power methods. RP-HPLC analysis revealed that the major phenolic acids and flavonoids in the EA were sinapic acid, ferulic acid, myricetin, and quercetin, suggesting that they were vital contributors to the antioxidant activity. These findings serve as an insight into the use of *M. saigonense* seeds as a potential source of natural antioxidants.

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