

# α-Glucosidase Inhibitory Activity of Phenolic Rich Extracts Obtained from the Seeds of *Melastoma saigonense* (Kuntze) Merr.

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Received: 15 July 2019;	Accepted: 6 September 2019;	Published online: 16 November 2019;	AJC-19648

The aim of this study was to perform a phytochemical analysis of *Melastoma saigonense* seed extracts and to determine their  $\alpha$ -glucosidase inhibitory activity. The extracts from seeds of *M. saigonense* indicated that the total phenolic content was in the range between 233.46 and 967.22 mg GAE/g DE, whereas the flavonoids content was in the range between 359.96 and 850.84 mg QE/g DE. The present study of antidiabetic inhibitory activity by *in vitro*  $\alpha$ -glucosidase revealed that the crude extracts using ethyl acetate (EA), butanol (BU) and final aqueous residue extracts (AQ) exhibited a strong  $\alpha$ -glucosidase inhibitory effect (IC<sub>50</sub> 4.42-11.95 µg/mL). The ethyl acetate and butanol extracts of seeds of *Melastoma saigonense* (Kuntze) Merr. were further fractionated by silica gel column chromatography into four fractions (EAF<sub>1</sub>–EAF<sub>4</sub>) and five fractions (BUF<sub>1</sub>–BUF<sub>5</sub>), respectively and their bioactivities were investigated. The nine fractions exhibited significant  $\alpha$ -glucosidase inhibitory activity (p < 0.05) with an IC<sub>50</sub> between 3.42-34.77 µg/mL which is less than the IC<sub>50</sub> for standard acarbose (IC<sub>50</sub> = 507.26 µg/mL). Among all the fractions, BUF<sub>1</sub> and EAF<sub>1</sub> exhibited high inhibitory activity against  $\alpha$ -glucosidase with BUF<sub>1</sub> showing the highest inhibitory activity (IC<sub>50</sub> = 3.42 µg/mL). The dominant phenolic acids were sinapic, gallic, ferrulic, syringic, gallic and caffeic acids and the prominent flavonoids were myricetin and quercetin. These findings suggest that the seeds of *M. saigonense* have potential as a source of antidiabetic agent (s).

Keywords: α-Glucosidase, Antidiabetic, Phenolic acids, Flavonoids, Melastoma saigonense.

#### **INTRODUCTION**

*Melastoma saigonense* (Kuntze) Merr. is a member of Melastomataceae family, commonly known in Thai as Khlong Khleng Yuan. It is a shrub with a height up to 3 m and found in many areas of Southeast Asia, including Thailand, Myanmar, Laos, Vietnam and Cambodia. The roots of *M. saigonense* have been used as a source of traditional medicines for relieving fever, stimulating appetite and improving the performance of the kidney and the liver. The fruit of *M. saigonense* are capsule, dry dehiscent fruit reveal the seeds embedded in a dark purple pulp, which can be eaten and has a sweet-sour taste similar to mulberry.

The Melastomataceae family includes eight species, which can be found in Thailand, including *M. cyanoides*, *M. imbricatum*, *M. malabathricum*, *M. pellegrinianum*, *M. minahassae*, *M. orientale*, *M. saigonense* and *M. sanguineum*. Only *Melastoma* 

malabathicum have been studied for phytochemicals. The studies revealed the presence of naringinin, quercetin, kaempferol, kaempferol-3-O-D-glucoside, and kaempferol-3-O-(2",6"-di-O-p-trans-coumaroyl)glucoside [1,2]. To the best of our knowledge, the bioactive evaluation of several crude extracts of Melastomataceae family showed numerous bioactivities such as anticancer [3], antibacterial [4], antiproliferative [5], hepatoprotective [6], antihyperlipidaemic [7] and antioxidant [4,7,8] activities. In addition, ethanolic extract of Melastoma malabathricum leaf significantly reduced blood glucose level in diabetic rats. However, there have been no studies of the phytochemical and bioactivities of Melastoma saigonense. We are concerned with the antidiabetic activity of seeds of *M. saigonense*, thus the aim of this study was to determine the  $\alpha$ -glucosidase inhibitory activity and phytochemical analysis of Melastoma saigonense seed extracts.

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#### **EXPERIMENTAL**

Phenolic standards used in this study included caffeic acid, p-coumaric acid, ferulic acid, gallic acid, p-hydroxybenzoic acid, protocatechuic acid, sinapic acid and syringic acid. Flavonoid standards were kaempferol, myricetin, quercetin and rutin. Acarbose,  $\alpha$ -glucosidase (EC 3.2.1.20) and *p*-nitrophenyl- $\alpha$ -D-glucopyranoside (*p*-NPG) were purchased from Sigma-Aldrich (St. Louis, USA). All other chemicals and reagents used in the study were of analytical grade. Column chromatography (CC) and thin layer chromatography (TLC) were performed over silica gel (< 0.063 mm) and silica gel 60 F<sub>254</sub>, respectively, obtained from Merck KGaA, Darmstadt, Germany. Absorbance measurements were made using a Perkin Elmer Lamda 35 spectrophotometer (Perkin-Elmer, USA). The analysis of phenolic acids and flavonoids present in extracts was performed by HPLC using Shimadzu LC-20AC pumps, a SPD-M20A diode array detector and chromatographic separations on a column Inetsil ODS-3, C18 (4.6 mm  $\times$  250 mm, 5  $\mu$ m) (Hichrom Limited, Berks, UK).

Plant materials, extraction and fractionation: The fruits of Melastoma saigonense were collected from Sakon Nakhon province, Thailand and the plant was identified botanically at the Botanical section of Walai Rukhavej Botanical Research Institute, Mahasarakham University, Thailand. The seeds (500 g) obtained from fresh fruit samples were extracted with methanol at room temperature (72 h) and then filtered. The filtrate was concentrated under reduced pressure using a rotary evaporator maintained at 40 °C to give a crude methanol extract. The crude extract was then dissolved in distilled water and partitioned in triplicate successively with hexane, ethyl acetate and *n*-butanol, respectively. All partitioned extracts were further concentrated to yield crude hexane (HE, 1.023 g), ethyl acetate (EA, 42.728 g) and butanol (BU, 18.182 g), together with the final aqueous residue extracts (AQ, 86.818 g). All extracts were stored in a refrigerator at 4 °C. The EA and BU extracts showed the strongest  $\alpha$ -glucosidase inhibitory activity and were selected for further fractionation. The active EA and BU extracts were fractionated consequently by column chromatography over silica gel (< 0.063 mm), eluted with solvent mixtures of different polarity and 50 mL of each fraction was collected continuously then monitored by TLC. Based on TLC analysis, four fractions designated EAF1-EAF4 were obtained from the EA extract, whereas five fractions designated BUF<sub>1</sub>-BUF<sub>5</sub> were obtained from the BU extract. The eluted fractions were evaporated using a rotary evaporator, condensed and then filtered through a membrane filter before being separated by HPLC.

**Determination of total phenolic content (TPC):** TPC was measured using Folin-Ciocalteu reagent with gallic acid used as the standard phenolic compound following Sulaiman *et al.* [9] method with some modification. Briefly, each diluted extract (170 L) was reacted with 83 L Folin-Ciocalteu reagent and after 5 min neutralized with 7.5 % (w/v) sodium carbonate after which the mixture was left to react for 2 h at room temperature. The absorbance of the resulting blue coloured solution was measured at 760 nm and the total phenolic content expressed as mg gallic acid equivalent per g dry weight extract (mg GAE/g DE).

**Determination of total flavonoid content (TFC):** The TFC was determined by a colorimetric assay according to the method of Zhishen *et al.* [10]. A 500  $\mu$ L plant sample was mixed with 100  $\mu$ L of NaNO<sub>2</sub> solution and the mixture allowed to stand for 6 min before 200  $\mu$ L of 10 % aluminium chloride solution was added. After 5 min, 500  $\mu$ L of 1 M NaOH was added to the mixture together with distilled water to bring the final volume to 1.5 mL. The absorbance was read at 510 nm and the results were expressed as mg quercetin equivalent per g dry weight extract (mg QE/g DE).

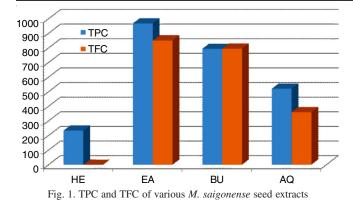
**α**-Glucosidase inhibition: The α-glucosidase inhibition activity of samples was determined using a slightly modified version of Kim *et al.* method [11]. A 200 µL of extract solution was added to 0.1 M sodium phosphate buffer (pH 6.9) and then mixed with 100 µL of α-glucosidase solution (0.08 U/mL) before being allowed to stand at 37 °C for 20 min. Then, 100 µL of 1 mM *p*-NPG (in phosphate buffer) was added and the reaction mixture was incubated at 37 °C for 20 min before being stopped by the addition of 450 µL of 0.2 M Na<sub>2</sub>CO<sub>3</sub>. The α-glucosidase activity was determined by measuring the *p*-nitrophenol released from p-NPG at 405 nm. Acarbose was used as a positive control and the IC<sub>50</sub> value was defined as the concentration of α-glucosidase inhibitor required to inhibit 50 % of its activity.

HPLC analysis of phenolic acids and flavonoids: The method which was followed has been described by Kaewseejan et al. [12]. The mobile phase was acetic acid pH 2.74 (solvent A) and acetonitrile (solvent B) delivered at a flow rate of 0.8 mL/min. The gradient elution was performed as follows: from 0 to 5 min a linear gradient from 5 to 9 % of solvent B; from 5 to 15 min a stable 9 % of solvent B; from 15 to 22 min a linear gradient from 9 to 11 % of solvent B; from 22 to 38 min a linear gradient from 11 to 18 % of solvent B; from 38 to 43 min a linear gradient from 18 to 23 % of solvent B; from 43 to 44 min a linear gradient from 23 to 90 % of solvent B; from 44 to 45 min a linear gradient from 90 to 80 % of solvent B; from 45 to 55 min, isocratic at 80 % of solvent B and from 55 to 60 min a linear gradient from 80 to 5 % of solvent B. A reequilibration period of 5 min with 5 % of solvent B was used between individual runs. The operating conditions were as follows: column temperature 38 °C, injection volume 20 µL and UV-diode array detector at 280 nm (phenolic acid) or 370 nm (flavonoids). Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with those of authentic standards using the external standard method.

### **RESULTS AND DISCUSSION**

Total phenolic content (TPC) of extracts (Fig. 1) obtained from *Melastoma saigonense* seeds ranged from 233.46 mg GAE/g DE (HE extract) to 967.22 mg GAE/g DE (EA extract). The TFC of the extracts ranged from 359.96 mg QE/g DE (AQ extract) to 850.84 mg QE/g DE (EA extract) and no flavonoid compounds were detected in the hexane extract. The results showed that the EA, BU and AQ extracts were rich in phenolics and flavonoids, which have been acknowledged to show significant  $\alpha$ -glucosidase inhibition [13,14].

 $\alpha$ -Glucosidase is a key enzyme for carbohydrate digestion and the inhibition of this enzyme has been shown to retard the



digestion and absorption of carbohydrates and consequently to inhibit the increase in postprandial glucose concentration. Thus,  $\alpha$ -glucosidase inhibitors can play a major role as chemotherapeutic agents for non-insulin dependent diabetes mellitus [15-19]. A literature search found no previously reported evidence for  $\alpha$ -glucosidase inhibitory activity of *M. saigonense* seed and so this is the first report on  $\alpha$ -glucosidase activity of this plant.

The  $\alpha$ -glucosidase inhibitory effect of the crude extract and its fractions were evaluated using  $\alpha$ -glucosidase obtained from yeast (Saccharomyces cerevisiae) and when using p-NPG as a substrate. The results illustrated that EA extract inhibited the highest inhibitory activity with the lowest IC<sub>50</sub> value of  $4.42 \pm 0.12 \,\mu$ g/mL, followed by the BU extract (IC<sub>50</sub> = 8.06 ±  $0.04 \,\mu g/mL$ ) and the AQ extract (IC<sub>50</sub> = 11.95 ± 0.30  $\mu g/mL$ ). Surprisingly, EA, BU and AQ extracts showed 40-100 times better  $\alpha$ -glucosidase inhibitory activity than the positive control acarbose (IC<sub>50</sub> = 507.26  $\pm$  10.87 µg/mL). Furthermore,  $\alpha$ -glucosidase inhibitory activity of EA extract was stronger than the BU and AQ extracts by factors of approximately 2 to 3, possibly because EA extract contained higher levels of phenolic compounds. According to the previous reports [19,20], phenolic compounds have been shown to possess the ability to bind to the active sites of  $\alpha$ -glucosidase thereby retarding the enzyme substrate reaction. In present study, the Pearson correlation coefficient (Table-1) showed that  $\alpha$ -glucosidase inhibitory potential of the extracts correlated strongly with their phenolic acid (r =0.931, p < 0.01) and flavonoid (r = 0.940, p < 0.01) contents. These results suggested that  $\alpha$ -glucosidase inhibition depends on the content of phenolics and flavonoids and thus the seeds of Melastoma saigonense are an abundant source of α-glucosidase inhibitor active compounds.

TABLE-1 PEARSON CORRELATION COEFFICIENTS (R) FOR RELATIONSHIPS BETWEEN TPC, TFC AND α-GLUCOSIDASE INHIBITION						
	TPC	TFC	α-Glucosidase inhibition			
TPC	1	0.953**	-0.931**			
TFC	-	1	-0.940**			
α-Glucosidase inhibition	_	-	1			
**Significant different at p < 0.01.						

The BUF<sub>1</sub> and EAF<sub>1</sub> showed the strongest  $\alpha$ -glucosidase inhibitory activity (IC<sub>50</sub> = 3.42 and 3.47 µg/mL, respectively) when compared to other fractions (Table-2). Moreover, the levels

TABLE-2
α-GLUCOSIDASE INHIBITORY ACTIVITY OF ETHYL
ACETATE EXTRACT, BUTANOL EXTRACT AND THEIR
FRACTIONS OBTAINED FROM M. saigonense SEEDS

Extracts and		α-Glucosidase inhibition
fractions		IC <sub>50</sub> (µg/mL)
Ethyl acetate (EA)		$4.42 \pm 0.12^{\rm f}$
fractions	$EAF_1$	$3.47 \pm 0.02^{\rm f}$
	$EAF_2$	$16.06 \pm 0.09^{\circ}$
	$EAF_3$	$34.77 \pm 0.08^{b}$
	$EAF_4$	$16.85 \pm 0.02^{\circ}$
Butanol (BU)		$8.06 \pm 0.04^{\circ}$
fractions	$BUF_1$	$3.42 \pm 0.01^{f}$
	$BUF_2$	$17.75 \pm 0.07^{\circ}$
	BUF <sub>3</sub>	$18.10 \pm 0.06^{\circ}$
	$BUF_4$	$13.66 \pm 0.06^{d}$
	$BUF_5$	$32.18 \pm 0.11^{b}$
Acarbose		$507.26 \pm 10.87^{a}$

All data are expressed as mean  $\pm$  SD (n = 3). Values in the same column with different superscript letters represent significant differences at p < 0.05.

of  $\alpha$ -glucosidase inhibition activity of BUF<sub>1</sub> and EAF<sub>1</sub> were 148 and 146 times higher than those of acarbose. Thus, BUF<sub>1</sub> and EAF<sub>1</sub> showed excellent  $\alpha$ -glucosidase inhibition activity, demonstrating that the compounds with the strongest  $\alpha$ -glucosidase inhibitory activity were selectively concentrated in BUF<sub>1</sub> and EAF<sub>1</sub> during the fractionation by silica gel column chromatography. Previous studies reported that  $\alpha$ -glucosidase inhibitory activity was increased due to the presence of phenolic chromophores [13,14], possibly because of the presence of more hydroxyl groups. Furthermore, the removal of hydroxyl groups in flavonoids has been shown to decrease  $\alpha$ -glucosidase inhibitory activity [17]. These findings suggest that phenolic compounds might be a major contributor to  $\alpha$ -glucosidase inhibition.

The composition and concentration of phenolic compounds were identified using HPLC and compared with authentic standards. The results (Tables 3 and 4) indicated the presence of sinapic acid, gallic acid, myricetin, ferulic acid, syringic acid, p-coumaric acid, caffeic acid and quercetin being the dominant polyphenolic compounds in the seeds of *M. saigonense*. These phenolic acids were detected in EA, BU and AQ extracts whereas EA extract showed the highest concentration of total phenolic acid followed by BU and AQ. The results in Table-3 revealed that the most prominent phenolic acid in BUF<sub>1</sub> and EAF<sub>1</sub> fractions was sinapic acid (871.20 and 533.83 µg/g DE, respectively), suggested that sinapic acid in the seeds of Melastoma saigonense might be responsible for its potent  $\alpha$ -glucosidase inhibitory capacity. The results have demonstrated that the type of phenolic acid as well as their chemical structure affect  $\alpha$ glucosidase inhibitory activity, which is in accordance with previous studies which reported that large structural diversity among different subclasses of phenolic compounds within the group affected their solubility, stability and bonding ability with  $\alpha$ -glucosidase [21].

As mentioned earlier, extracts obtained from the seeds of *Melastoma saigonense* seem to have higher levels of flavonoids than phenolic acids. Table-4 shows that the major flavonoids present in the fractions were myricetin, quercetin and kaempferol. Previous studies have reported that myricetin and kaempferol have a variety of bioactivities, such as anti-inflammatory, anti-

 TABLE-3

 COMPOSITIONS OF PHENOLIC ACIDS IN DIFFERENT FRACTIONS OBTAINED FROM M. saigonense SEEDS

	Phenolic fraction content (µg/g DE)								
Fractions	Gallic acid	Protocate- chuic acid	<i>p</i> -Hydroxy benzoic	Syringic acid	Caffeic acid	<i>p</i> -Couma- ric	Ferulic acid	Sinapic acid	Total
EA	493.85 ± 0.84°	84.01 ± 1.34 <sup>h</sup>	$78.19 \pm 0.05^{i}$	235.12 ± 1.77°	123.89 ± 2.12 <sup>g</sup>	$129.12 \pm 0.06^{\rm f}$	$477.47 \pm 0.18^{d}$	668.35 ± 0.51 <sup>b</sup>	$2,290.00 \pm 6.87^{a}$
EAF <sub>1</sub>	281.97 ± 2.12°	53.43 ± 0.29 <sup>h</sup>	$62.11 \pm 1.10^{g}$	$211.38 \pm 0.02^{d}$	$123.05 \pm 2.11^{\text{f}}$	$67.23 \pm 1.05^{g}$	201.58 ± 1.46 <sup>e</sup>	871.20 ± 4.73 <sup>b</sup>	$1,871.95 \pm 12.88^{a}$
EAF <sub>2</sub>	$103.20 \pm 1.55^{d}$	$63.85 \pm 0.18^{\rm f}$	$29.22 \pm 0.62^{i}$	43.99 ± 3.31 <sup>h</sup>	91.47 ± 0.06 <sup>e</sup>	$57.05 \pm 0.26^{g}$	164.83 ± 0.29 <sup>c</sup>	193.85 ± 0.35 <sup>b</sup>	$747.46 \pm 6.62^{a}$
EAF <sub>3</sub>	$171.09 \pm 0.05^{b}$	ND	ND	105.70 ± 0.02°	ND	$42.87 \pm 0.41^{d}$	105.51 ± 0.81°	34.15 ± 0.99 <sup>e</sup>	$459.32 \pm 2.28^{a}$
$EAF_4$	276.34 ± 0.47°	ND	87.91 ± 0.55°	$132.98 \pm 0.81^{d}$	83.85 ± 0.04 <sup>e</sup>	$65.18 \pm 0.62^{\rm f}$	393.85 ± 0.84 <sup>b</sup>	393.85 ± 3.13 <sup>b</sup>	1,433.96 ± 6.46 <sup>a</sup>
BU	$265.31 \pm 0.69^{d}$	65.90 ± 0.41 <sup>h</sup>	$65.62 \pm 0.86^{h}$	115.09 ± 2.67 <sup>f</sup>	$87.22 \pm 1.02^{g}$	135.45 ± 2.01°	409.21 ± 1.64 <sup>c</sup>	485.54 ± 3.34 <sup>b</sup>	1,629.34 ± 12.64 <sup>a</sup>
BUF <sub>1</sub>	129.12 ± 1.77 <sup>f</sup>	45.36 ± 2.15 <sup>i</sup>	$86.88 \pm 0.69^{h}$	196.34 ± 0.20°	$103.16 \pm 0.72^{g}$	$309.41 \pm 3.28^{\circ}$	$235.18 \pm 0.92^{d}$	533.83 ± 1.71 <sup>b</sup>	1,639.28 ± 11.44 <sup>a</sup>
BUF <sub>2</sub>	67.33 ± 0.76 <sup>g</sup>	$77.15 \pm 2.10^{\text{f}}$	$84.01 \pm 0.24^{\rm f}$	129.72 ± 0.46 <sup>e</sup>	135.02 ± 0.81°	235.71 ± 2.64 <sup>d</sup>	405.14 ± 3.34°	493.70 ± 3.73 <sup>b</sup>	1,627.78 ± 14.08 <sup>a</sup>
BUF <sub>3</sub>	$95.97 \pm 0.40^{d}$	ND	ND	75.69 ± 0.86 <sup>e</sup>	$65.10 \pm 0.41^{\text{f}}$	122.05 ± 2.51°	210.09 ± 3.12 <sup>b</sup>	$215.11 \pm 0.20^{b}$	784.01 ± 7.50 <sup>a</sup>
$\mathrm{BUF}_4$	$78.19 \pm 0.06^{\rm f}$	ND	ND	$121.62 \pm 0.86^{d}$	$69.01 \pm 0.36^{g}$	$88.52 \pm 1.45^{\circ}$	$152.02 \pm 0.67^{\circ}$	$185.20 \pm 1.68^{b}$	$694.56 \pm 5.08^{a}$
BUF <sub>5</sub>	$77.47 \pm 0.18^{\rm f}$	87.21 ± 3.78°	$49.12 \pm 0.73^{g}$	$83.02 \pm 0.55^{\rm f}$	$75.69 \pm 0.06^{\rm f}$	$135.29 \pm 0.86^{d}$	279.21 ± 3.81°	654.81 ± 2.32 <sup>b</sup>	$1,441.82 \pm 12.29^{a}$
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Data are expressed as the mean  $\pm$  SD (n=3). Values in the same row with the same superscript letters represent significant differences at the p < 0.05 confidence level.

TABLE-4           CONTENT OF FLAVONOIDS IN DIFFERENT FRACTIONS OBTAINED FROM M. saigonense SEEDS							
Fractions —	Flavonoid fraction content (µg/g DE)						
	Rutin	Myricetin	Quercetin	Kaempferol	Total		
EA	$252.10 \pm 0.47^{\circ}$	$2308.06 \pm 4.96^{b}$	$2162.65 \pm 6.83^{\circ}$	$393.15 \pm 0.41^{d}$	5,115.96 ± 12.67 <sup>a</sup>		
$EAF_1$	$197.37 \pm 0.98^{d}$	$2124.18 \pm 1.65^{\circ}$	$2668.85 \pm 0.35^{b}$	$85.62 \pm 0.52^{\circ}$	$5,076.02 \pm 3.50^{\text{a}}$		
$EAF_2$	$160.65 \pm 0.18^{d}$	1050.69 ± 3.13 <sup>b</sup>	$718.30 \pm 1.21^{\circ}$	$126.01 \pm 0.55^{\circ}$	$2,055.65 \pm 5.07^{\text{a}}$		
EAF <sub>3</sub>	$92.23 \pm 0.62^{\circ}$	$513.85 \pm 0.84^{\text{ d}}$	656.47 ± 2.63 <sup>b</sup>	$518.61 \pm 0.45^{\circ}$	$1,781.16 \pm 4.54^{a}$		
$EAF_4$	$120.60 \pm 0.58^{\circ}$	2153.38 ± 3.51 <sup>b</sup>	$1393.25 \pm 0.24^{\circ}$	$417.32 \pm 2.39^{d}$	$4,084.55 \pm 6.72^{a}$		
BU	$232.32 \pm 0.86^{\circ}$	$875.69 \pm 0.86^{\circ}$	1035.34 ± 3.86 <sup>b</sup>	$336.29 \pm 1.46^{d}$	$2,479.64 \pm 7.04^{a}$		
BUF <sub>1</sub>	$201.78 \pm 1.65^{d}$	$508.01 \pm 0.45^{\circ}$	1231.71 ± 3.78 <sup>b</sup>	$185.24 \pm 4.23^{\circ}$	$2,126.74 \pm 10.11^{a}$		
BUF <sub>2</sub>	$172.45 \pm 1.47^{d}$	$398.64 \pm 2.02^{\circ}$	1185.39 ± 3.28 <sup>b</sup>	ND	$1,756.48 \pm 6.77^{a}$		
BUF <sub>3</sub>	ND	$402.97 \pm 3.34^{\circ}$	$613.75 \pm 2.78^{b}$	$135.61 \pm 0.46^{d}$	$1,152.33 \pm 6.58^{a}$		
BUF <sub>4</sub>	$179.71 \pm 0.67$ <sup>d</sup>	$285.54 \pm 1.28^{\circ}$	$346.29 \pm 1.61^{b}$	ND	$811.54 \pm 3.56^{a}$		
BUF <sub>5</sub>	ND	$617.15 \pm 0.67$ °	654.65 ± 2.32 <sup>b</sup>	$215.20 \pm 1.52^{d}$	$1,487.00 \pm 4.51^{a}$		

Data are expressed as mean  $\pm$  SD (n = 3). Values in the same row with different superscript letters represent significant differences at p < 0.05.

cancer, antiviral, antioxidant and antidiabetic activities. The bioactivities were established due to the chemical structures and number of hydroxyl groups present in the flavonoids detected [12]. According to Wang et al. [22], hydroxyl group at the 3-position on A-ring of the flavonoids and a number of hydroxyl groups attached to C-ring play important roles in the  $\alpha$ -glucosidase inhibitory activity. Table-4 showed that EAF<sub>1</sub> appeared to have the highest flavonoid content (5,076.02 µg/ g DE), followed by EAF<sub>4</sub> (4084.55  $\mu$ g/g DE), BUF<sub>1</sub> (2126.74 μg/g DE), EAF<sub>2</sub> (2055.65 μg/g DE), EAF<sub>3</sub> (1781.16 μg/g DE), BUF<sub>2</sub> (1756.48 µg/g DE), BUF<sub>5</sub> (1487.00 µg/g DE), BUF<sub>3</sub> (1152.33  $\mu$ g/g DE) and BUF<sub>4</sub> (811.54  $\mu$ g/g DE). The major flavonoids in EAF1 were quercetin and myricetin, which accounted for approximately 53 and 42 % of the total flavonoid content as determined by HPLC, respectively. Tan et al. [23] reported that flavonoids have higher enzyme inhibition activity than phenolic acids, especially with regards to  $\alpha$ -glucosidase,

and showed that myricetin was a highly potent  $\alpha$ -glucosidase inhibitor followed by epicatechin, catechin and quercetin. Since this study has revealed that BUF<sub>1</sub> and EAF<sub>1</sub> expressed the strongest  $\alpha$ -glucosidase inhibition and had higher concentrations of myricetin and quercetin than other fractions, the compounds responsible for  $\alpha$ -glucosidase inhibition might be myricetin and quercetin.

#### Conclusion

The present *in vitro* study investigated the potential antidiabetic activity of extracts obtained from the seeds of *Melastoma saigonense*, focusing on their capacity for  $\alpha$ -glucosidase inhibition. The results demonstrated that phytochemicals in the seed extracts obtained from *Melastoma saigonense* have significant antidiabetic activity, directly related to the total amounts of phenolics and flavonoids present. Present study is the first to report the antidiabetic activity of extracts obtained from the seeds from *M. saigonense* and suggests that glucose lowering effect of the plant extracts is due to inhibition of the digestive enzyme  $\alpha$ -glucosidase. Thus, the abundance of phenolics in seeds obtained from *M. saigonense* could be used as a source of a natural antidiabetic.

## ACKNOWLEDGEMENTS

The authors thank Departments of Chemistry, Faculty of Science at Mahasarakham University and Udon Thani Rajabhat University in Thailand for providing the facilities to carry out this work. Thanks are due to Dr. Sutthira Khumkratok for helping in the identification of plant *Melastoma saigonense*.

#### **CONFLICT OF INTEREST**

The authors declare that there is no conflict of interests regarding the publication of this article.

#### REFERENCES

- D. Susanti, H.M. Sirat, F. Ahmad, R.M. Ali, N. Aimi, and M. Kitajima, Food Chem., 103, 710 (2007);
- https://doi.org/10.1016/j.foodchem.2006.09.011. 2. D. Susanti, H.M. Sirat, F. Ahmad and R.M. Ali, *J. Ilmiah Farm.*, **5**, 1
- (2008) (In Indonesian).
  N.A. Roslen, N.A.M. Alewi, H. Ahamada and M.S.B.A. Rasad, *Asian Pac J. Trop Biomed.*, 4, 545 (2014);
- https://doi.org/10.12980/APJTB.4.2014C658.
   Z.A.A. Alnajar, M.A. Abdulla, H.M. Ali, M.A. Alshawsh and A.H.A. Hadi, *Molecules*, **17**, 3547 (2012);
- https://doi.org/10.3390/molecules17033547.
  Z.A. Zakaria, M.S. Rofiee, A.M. Mohamed, L.K. Teh and M.Z. Salleh, J. Acupunct. Meridian Stud., 4, 248 (2011);
- https://doi.org/10.1016/j.jams.2011.09.016.
  F.H. Kamisan, F. Yahya, N.A. Ismail, S.S. Din, S.S. Mamat, Z. Zabidi, W.N.W. Zainulddin, N. Mohtarrudin, H. Husain, Z. Ahmad and Z.A. Zakaria, *J. Acupunct. Meridian Stud.*, 6, 52 (2013); https://doi.org/10.1016/j.jams.2012.08.002.
- K. Balamurugan, A. Nishanthini and V.R. Mohan, Asian Pac. J. Trop. Biomed., 4, S442 (2014); https://doi.org/10.12980/APJTB.4.2014C122.

 L. Fu, B.-T. Xu, X.-R. Xu, X.-S. Qin, R.-Y. Gan and H.-B. Li, *Molecules*, 15, 8602 (2010);

https://doi.org/10.3390/molecules15128602.

- S.F. Sulaiman, N.A.M. Yusoff, I.M. Eldeen, E.M. Seow, A.A.B. Sajak, Supriatno and K.L. Ooi, *J. Food Compos. Anal.*, 24, 1 (2011); https://doi.org/10.1016/j.jfca.2010.04.005.
- J. Zhishen, T. Mengcheng and W. Jianming, *Food Chem.*, 64, 555 (1999); <u>https://doi.org/10.1016/S0308-8146(98)00102-2</u>.
- 11. Y.M. Kim, M.H. Wang and H.I. Rhee, *Carbohydr. Res.*, **339**, 715 (2004); https://doi.org/10.1016/j.carres.2003.11.005.
- N. Kaewseejan, V. Sutthikhum and S. Siriamornpun, J. Funct. Foods, 12, 120 (2015);

https://doi.org/10.1016/j.jff.2014.11.001.

- B. Jabeen, N. Riaz, M. Saleem, M.A. Naveed, M. Ashraf, U. Alam, H.M. Rafiq, R.B. Tareen and A. Jabbar, *Phytochemistry*, 96, 443 (2013); <u>https://doi.org/10.1016/j.phytochem.2013.09.015</u>.
- E. Thilagam, B. Parimaladeve, C. Kumarappan and S.C. Mandal, J. Acupunct. Meridian Stud., 6, 24 (2013); https://doi.org/10.1016/j.jams.2012.10.005.
- D. De, T.K. Bera, K.M. Ali, S. Mandal, B. Barik, D. Ghosh, *Biomark. Genom. Med.*, 5, 48 (2013); https://doi.org/10.1016/j.gmbhs.2013.04.003.
- F. Ferreres, J. Vinholes, A. Gil-Izquierdo, P. Valentão, R.F. Gonçalves and P.B. Andrade, *Food Chem.*, **136**, 1390 (2013); https://doi.org/10.1016/j.foodchem.2012.09.089.
- A.W. Indrianingsih, S. Tachibana, R.T. Dewi and K. Itoh, *Asian Pac. J. Trop. Biomed.*, **5**, 748 (2015); https://doi.org/10.1016/j.apjtb.2015.07.004.
- Y. Wang, S. Huang, S. Shao, L. Qian and P. Xu, *Ind. Crops Prod.*, 37, 520 (2012);
- https://doi.org/10.1016/j.indcrop.2011.07.031.
  T. Wang, X. Li, B. Zhou, H. Li, J. Zeng and W. Gao, *J. Funct. Foods*, 13, 276 (2015);
- https://doi.org/10.1016/j.jff.2014.12.049. 20. R. Ramu, P.S. Shirahatti, F. Zameer, L.V. Ranganatha and M.N.N.
- Prasad, S. Afr. J. Bot., **95**, 54 (2014); https://doi.org/10.1016/j.sajb.2014.08.001.
- A. Wojdylo, P. Nowicka, Á.A. Carbonell-Barrachina and F. Hernández, J. Funct. Foods, 25, 421 (2016); https://doi.org/10.1016/j.jff.2016.06.015.
- 22. H. Wang, Y. Du and H. Song, *Food Chem.*, **123**, 6 (2010); https://doi.org/10.1016/j.foodchem.2010.03.088.
- Y. Tan, S.K.C. Chang and Y. Zhang, *Food Chem.*, 214, 259 (2017); https://doi.org/10.1016/j.foodchem.2016.06.100.