ความสามารถในการต้านอนุมูลอิสระ และฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดส ของสารสกัดน้ำจากต้นเฉาก๊วย

Antioxidant Activity and α-Glucosidase Inhibitory Activity of *Mesona chinensis* Aqueous Extract

อรสา สุริยาพันธ์[1*], กันต์กนิษฐ์ ตั้งสถิตย์กุลชัย[1], ศวิตา จิวจินดา[1],
ปัทมพรรณ โลมะรัตน์[1] และ วิลาสินี หิรัญพานิช ซาโตะ[2]

Orasa Suriyaphan[1*], Kankanit Tangsathitkulchai[1], Savita Chewchinda[1],
Pattamapan Lomarat[1] and Vilasinee Hirunpanich Sato[2]

[1] *ภาควิชาอาหารเคมี คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ประเทศไทย*

[2] *ภาควิชาเภสัชวิทยา คณะเภสัชศาสตร์ มหาวิทยาลัยมหิดล ประเทศไทย*

[1] *Department of Food Chemistry, Faculty of Pharmacy, Mahidol University, Thailand*

[2] *Department of Pharmacology, Faculty of Pharmacy, Mahidol University, Thailand*

Received : 1 June 2023
Revised : 7 June 2023
Accepted : 14 June 2023

บทคัดย่อ

ในประเทศไทยใช้ต้นเฉาก๊วย (*Mesona chinensis*) แห้งเป็นวัตถุดิบหลักในการผลิตวุ้นเฉาก๊วย ได้มีรายงานว่าสารสกัดน้ำจากต้นเฉาก๊วย (*Mesona chinensis* aqueous extract, MCAE) มีสารที่มีฤทธิ์ต้านภาวะน้ำตาลในเลือดสูงที่ดี ความเข้าใจที่ดีขึ้นเกี่ยวกับการผลิตและฤทธิ์ทางชีวภาพของสารสกัดน้ำจากต้นเฉาก๊วยย่อมมีส่วนช่วยในการพัฒนาส่วนผสมฟังก์ชันและเครื่องดื่มฟังก์ชันชนิดใหม่ ดังนั้น ในงานวิจัยนี้ได้ศึกษาอิทธิพลของแหล่งปลูกของต้นเฉาก๊วยต่อปริมาณสารออกฤทธิ์ทางชีวภาพ ความสามารถในการต้านอนุมูลอิสระและฤทธิ์ลดระดับน้ำตาลของสารสกัดน้ำจากต้นเฉาก๊วยจำนวน 4 ตัวอย่างที่เตรียมจากต้นเฉาก๊วยแห้งนำเข้าจากประเทศจีน (2 ตัวอย่าง) ประเทศเวียดนาม และประเทศอินโดนีเซีย การวิเคราะห์ข้อมูลทางสถิติพบว่า แหล่งปลูกของต้นเฉาก๊วยแห้งมีผลต่อปริมาณสารฟีนอลิกทั้งหมด ปริมาณสารฟลาโวนอยด์ทั้งหมด ฤทธิ์ต้านอนุมูลอิสระดีพีพีเอช และฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดส ของสารสกัดน้ำจากต้นเฉาก๊วยอย่างมีนัยสำคัญทางสถิติ (p<0.05) สารสกัดน้ำจากต้นเฉาก๊วยมีปริมาณสารฟีนอลิกทั้งหมดในปริมาณค่อนข้างสูง(68.63-133.27 mg GAE/g) แต่มีปริมาณสารฟลาโวนอยด์ทั้งหมดในปริมาณที่ต่ำมาก(3.49-5.83 mg QE/g) สารสกัดน้ำจากต้นเฉาก๊วยมีฤทธิ์ต้านอนุมูลอิสระดีพีพีเอชที่ดี (IC_{50} = 17.54-37.98 μg/mL) และมีฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดสที่ดีมาก (IC_{50} = 4.77-26.86 μg/mL) การศึกษาจลนพลศาสตร์ของการยับยั้งการทำงานเอนไซม์แอลฟา-กลูโคซิเดสของสารสกัด พบว่าฤทธิ์การยับยั้งแปรผันตามความเข้มข้นของสารสกัดน้ำจากต้นเฉาก๊วย การสร้างกราฟไลน์วีเวอร์-เบิร์กแสดงให้เห็นว่าการยับยั้งการทำงานเอนไซม์ของสารสกัดน้ำจากต้นเฉาก๊วยเป็นการยับยั้งแบบไม่แข่งขันที่มีค่าคงที่ของการยับยั้ง(K_i) เท่ากับ 2.67 μg /mL.

คำสำคัญ : สารสกัดน้ำจากต้นเฉาก๊วย ; ความสามารถในการต้านอนุมูลอิสระ ; ฤทธิ์ยับยั้งเอนไซม์แอลฟา-กลูโคซิเดส ; การยับยั้งแบบไม่แข่งขัน

Abstract

Mesona chinensis is raw material for the production of a black grass jelly in Thailand. *Mesona chinensis* aqueous extract (MCAE) has been reported as a promising anti-hyperglycemia agent. The better understanding of MCAE on its production and biological activities would facilitate the development of a new functional ingredient and beverage. Thus, the effects of botanical source on bioactive compounds, antioxidant activity and hypoglycemic activity of MCAE were investigated. In this present study, we prepared four MCAEs from *M. chinensis* plants imported from China (2 sources), Vietnam, and Indonesia. Based on data analysis, botanical source of *M. chinensis* significantly governed total phenolic content, total flavonoids content, DPPH radical scavenging activity and α-glucosidase inhibition activity of MCAE (p<0.05). The results showed that MCAEs possessed considerable amount of total phenolics (68.63-133.27 mg GAE/g) and trace amount of total flavonoids (3.49-5.83 mg QE/g). MCAEs exhibited potent antioxidant (IC_{50} = 17.54-37.98 μg/mL) and efficient inhibitor against alpha-glucosidase (IC_{50} = 4.77-26.86 μg/mL). The enzyme kinetics data revealed that MCAE inhibited α-glucosidase activity in a concentration-dependent manner. Moreover, the Lineweaver-Burk plot elucidated that the inhibition type of α-glucosidase activity by MCAE was a noncompetitive manner with the inhibitory constant (K_i) of 2.67 μg/mL.

Keywords : *Mesona chinensis* aqueous extract ; Antioxidant activity ; alpha-glucosidase inhibitory activity ; noncompetitive manner

***Correspondence to E-mail** : orasa.sur@mahidol.ac.th

Introduction

Mesona chinensis is an annual plant belonging to Labiatae family widely found in Asia. In addition, this herbaceous plant is an economically agriculture herb cultivated mainly in China, Indonesia, and Vietnam. It has been used as raw material for the production of Black grass jelly in Thailand. For traditional Chinese treatment, *M. chinensis* has been used to alleviate diabetes, hypertension, heatstroke, and muscle joint pain (Hung & Yen, 2002; Huang *et al.*, 2011; Adisakwattana *et al.*, 2014). Several studies have discovered various promising pharmacological properties of *M chinensis* aqueous extract (MCAE). For instance, antioxidant (Hung & Yen, 2002), anti-inflammatory (Huang *et al.*, 2011), antihypertensive (Yeh *et al.*, 2009), hypolipidemic (Huang *et al.*, 2016), renal protective (Yang *et al.*, 2008), and hypoglycemic activities (Chusak *et al.*, 2014; Sasmita *et al.*, 2017; Lim *et al.*, 2018) were of great interest. Recently, MCAE has been become the outstanding anti-hyperglycemia agent due to its abilities to decrease postprandial plasma glucose level and to enhance antioxidant defense status in overweight people who consumed high carbohydrate meal (Chusak *et al.*, 2014).

Diabetes mellitus (DM) is a metabolic disorder characterized by high plasma glucose level. The prevalence of overweight and obesity are considered as significant factors involved in the onset of type 2 diabetes mellitus (T2DM) (Stefano *et al.*, 2018). Patients with T2DM have insulin resistance initiating the accumulation of glucose in the blood. The absorption of glucose in the intestinal lumen is virtually regulated by brush border hydrolases or α-glucosidase activities. These enzymes release one glucose molecule from oligosaccharide or hydrolyze disaccharide to monosaccharide. The liberated glucoses are then transported across epithelium cells to bloodstream (Stefano *et al.*, 2018). Thus, the inhibition of intestinal α-glucosidase activities can prevent or delay surge of plasma glucose level after meal consumption. Up to date, acarbose, voglibose and miglitol are clinically approved α-glucosidase inhibitor drugs for patients with T2DM (He *et al.*, 2015). However, some adverse side effects of anti-diabetic drugs, such as flatulence, nausea, abdominal pain, diarrhea, and liver disorders, have been reported (Dhameja & Gupta, 2019). To minimize noncompliance, there has been the great demand on searching for α-glucosidase inhibitors from natural resources such as herbs, spices, and food plants (Yin *et al.*, 2014; Stefano *et al.*, 2018; Seetaloo *et al.*, 2019). Furthermore, the development of high carbohydrate food products with natural ingredients that are capable of inhibiting α-glucosidase during digestion is substantial increasing of interest.

The better understanding of MCAE on its production and biological activities (i.e., antioxidant activity, hypoglycemic activity) would help to develop new healthy herbal drinks or functional foods containing high quality of MCAE. Hence, the main objectives of this study were 1) to determine effects of botanical source on bioactive compounds, antioxidant activity, α-glucosidase inhibitory activity of MCAE and 2) to characterize kinetics of α-glucosidase inhibition by MCAE.

Methods

Acarbose, *p*-nitrophenyl (PNP), 4-nitrophenyl α-D-glucopyranoside (*p*-NPG), α-glucosidase from *Saccharomyces cerevisiae*, gallic acid, quercetin were purchased from Sigma-Aldrich Chemical Company (USA). Aluminium chloride hexahydrate, sodium carbonate and L-ascorbic acid were purchased from Ajax Finechem Laboratory Chemicals (Australia). Folin-Ciocalteu's phenol reagent was purchased from Merck (Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was purchased from Alfa Aesar (USA). Methanol was purchased from RCI Labscan (Ireland)

1. Preparation of Mesona chinensis aqueous extract (MCAE)

Dried *M. chinensis* plants were kindly provided and identified their botanical sources by local well-established black grass jelly entrepreneur in Thailand (Pattanapoonphol Co., Ltd., Bangkok). Four samples of dried *M. chinensis* plants were commercially imported items from China (2 sources), Indonesia and Vietnam. Prior to the extraction step, all dried plants were washed to remove sand and debris with tap water for 5 min. Dampened plants were manually squeezed water out prior to drying process. They were dried in tray dryer at 50°C for 7-12 hours (ca.12% final moisture content). Then, the dried explants were cut into small pieces and were pulverized into powder using a grinder. The powder was then sieved through an 18-mesh sieve and stored in a well-sealed plastic bag.

All extractions were conducted by heating dried *M. chinensis* explant (20 g) with water (400 mL) at 80-95 °C in slow cookers for 2 hours (Yang *et al.*, 2008) and re-extracted 2 times. The pooled extract was consecutively filtered through cheesecloth, Whatman filter paper no. 4 and no. 1. The obtained filtrate was then concentrated by a rotary evaporator at 45-50 °C under reduced pressure (35-72 mbar). Finally, the concentrated extract was dried at -50 ºC by a lyophilizer (Martin Christ, Model Alpha 1-4 LD plus, Germany) into powder form (Yen *et al.*, 2001). The dried powder of MCAE was weighted to determine the total soluble components (Yen *et al.*, 2001). The percentage of yield was then calculated as total weight of MCAE per 100 grams of dried explant. The obtained MCAEs were kept in an amber bottle and stored in the freezer (-20 ºC).

2. Determination of bioactive compounds of MCAEs

2.1 Determination of total phenolic content (TPC)

The concentration of phenolic compounds was measured according to the method described by Vongsak *et al.* (2013) with some modifications. MCAE was re-dissolved in deionized water to obtain test solution with 1,000 μg MCAE/mL. The test solution (20 μL) was mixed with 50 μL of diluted Folin-Ciocalteu reagent (1:10 with deionized water) in a 96 well plate. The mixture was allowed to react for 3 minutes. Then, 80 μL of 7.5% (w/v) sodium carbonate solution was added. The mixture was allowed to stand for a further 120 minutes in the dark. The absorbance was then measured at 765 nm using microplate reader (Tecan, USA). The same procedure was repeated for the standard solution of gallic acid. The calibration curve was constructed by plotting the concentration of gallic acid (X, μg/mL) against absorbance at 765 nm (Y). The obtained equation was as follows, $Y=0.0058X+0.0109$ ($R^2=0.9993$). All

determinations were performed in triplicate. The result was expressed as mg of gallic acid equivalent per g extract (mg of GAE/g of MCAE).

2.2 Determination of total flavonoid content (TFC)

The concentration of flavonoids was determined using aluminum chloride colorimetric method (Vongsak *et al.*, 2013). MCAE was re-dissolved in deionized water to obtain test solution with 500 μg MCAE/mL. The test solution (100 μL) was mixed with 100 μL of 2% (w/v) aluminum chloride solution in a 96 well plate. The mixture was allowed to stand for 10 minutes. The absorbance was then measured at 415 nm using microplate reader. The same procedure was repeated for the standard solution of quercetin. The calibration curve was constructed by plotting the concentration of quercetin (X, μg/mL) against absorbance at 415 nm (Y). The obtained equation was as follows, $Y=0.0313X+0.0016$ ($R^2=0.9999$). All determinations were performed in triplicate. The result was expressed as mg of quercetin equivalent per g extract (mg of QE/g of MCAE).

3. Determination of biological activity of MCAEs

3.1 Determination of DPPH radical scavenging activity

The free radical scavenging activity was determined according to the method of Sithisarn *et al.* (2015) with some modifications. MCAE was re-dissolved in deionized water to obtain stock test solution with 1,000 μg MCAE/mL. Then, the serial dilutions were made to obtain working test solutions with concentration of 500, 250, 125, 62.5, 31.2, 15.6 and 7.8 μg MCAE/mL. One hundred microliters of working test solutions were mixed with 100 μL of 152 μM DPPH solution in methanol in a 96 well plate. The mixture was incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using microplate reader. The control contained all reagents except MACE. The percentage of scavenging was calculated as following,

$$\% \text{ scavenging} = [(A_{control}-A_{sample})/A_{control}] \times 100 \quad (1)$$

Then the IC_{50} values were calculated from the plot of % scavenging versus concentration of MCAE. The IC_{50} value was defined as the MCAE concentration required to inhibit 50% of DPPH radical. Ascorbic acid was used as positive control and its average IC_{50} value was determined to be 3.41±0.13 μg/mL.

3.2 Determination of in vitro α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity was assessed using the method of Supasuteekul *et al.* (2016). Briefly, the α-glucosidase and substrate (*p*NPG) solutions were dissolved in phosphate buffer (pH 6.9, 0.1M) to obtain enzyme activity of 0.1 Unit/mL and concentration of *p*NPG at 2 mM, respectively. The MCAE solution was prepared at four levels of concentration (1.25-30 μg/mL). The α-glucosidase solution (40 μL) was premixed with 10 μL of each concentration of the MCAE solution in 96-well plate. The mixture was pre-incubated at 37 °C for 10 minutes. Then, the substrate solution (50 μL) was added to start the reaction. The mixture was further incubated at 37 °C for

20 minutes. Finally, the reaction was stopped by adding 100 μL of 1 M sodium carbonate solution. The absorbance was measured at 405 nm using a fluorescence microplate reader (BMG Labtech, Germany). The α-glucosidase activity was determined by measuring the release of p-nitrophenol (PNP) from *p*NPG at 405 nm. The percentage of α-glucosidase inhibitory activity was calculated as following:

$$\% \text{ inhibitory activity} = [(A_{control}-A_{controlblank})-(A_{sample}-A_{sampleblank})/(A_{control}-A_{controlblank}] \times 100 \quad (2)$$

Where,

$A_{control}$ = absorbance of the enzyme (40 μL)+deionized water (10 μL)+substrate solution (50 μL)

$A_{controlblank}$ = absorbance of the enzyme (40 μL)+deionized water (10 μL)+phosphate buffer (50 μL)

A_{sample} = absorbance of the enzyme (40 μL)+MCAE solution (10 μL)+substrate solution (50 μL)

$A_{sampleblank}$ = absorbance of the enzyme (40 μL)+MCAE solution (10 μL)+phosphate buffer (50 μL)

The inhibition curve was then plotted between percentages of α-glucosidase inhibitory activity and MCAE concentrations to determine its IC_{50} value. The IC_{50} value was defined as the MCAE concentration required to inhibit 50% α-glucosidase activity. Acarbose was used as positive control and its average IC_{50} value was determined to be 414±40.39 μg/mL.

4. Kinetics of α-glucosidase inhibition analysis

The kinetic study of enzyme inhibition of MCAE was conducted according to method of the Manoka *et al.* (2016) with some modifications. Briefly, the standard curve of *p*-nitrophenol (PNP, product) was constructed by plotting the absorbance (Y) at 405 nm versus its concentration (X, 0.025-0.50 μmol/mL). The obtained standard equation was Y = 44.181X+0.022, R^2 = 0.9995. The MCAE solution was prepared at three levels of concentration (2.5, 5 and 7.5 μg/mL). The substrate (*p*NPG) solution was prepared at five different concentrations (0.25-4mM). The determination of α-glucosidase inhibitory activity was performed as aforementioned. Each MCAE solution was prepared for 2 sets of reactions (t_0 and t_{20}). Reaction at 0 minute (t_0) was represented the reaction was terminated immediately after addition of substrate solution. Reaction at 20 minutes (t_{20}) was designated as the reaction was stopped after 20 minutes incubation. The absorbance obtained from the experiments at t_{20}-t_0 and the standard curve (Y value) was used to calculate the amount of produced PNP (X value). The velocity of the reaction (*v*, μmol/min) was calculated by dividing the amount of product (PNP) by time. The reaction time was 20 minutes.

The effect of substrate concentration on reaction velocity can be mathematically expressed using Michaelis-Menten equation, which is as follows;

$$v = (V_{max} * [S])/ (K_m + [S]) \quad (3)$$

where v is the initial rate with substrate concentration equal to [S], V_{max} is the maximum rate, and K_m is the Michaelis-Menten constant for the specific substrate (Blanco & Blanco, 2017).

A Lineweaver-Burk plot was generated by plotting reciprocal of the substrate concentration (1/[S]) and reciprocal of the reaction velocity (1/*v*). Subsequently, the enzyme binding constant (Michaelis-Menten constant, K_m), the maximum rate of reaction with enzyme (V_{max}) and the inhibition constant (K_i) were determined by using Microsoft excel 2010 with Solver add-in (Chewchinda *et al*., 2021).

5. Statistical analysis

All experiments were performed in triplicate. The experimental data were analyzed using analysis of variance (ANOVA) and expressed as the mean ± standard deviation (SD). When ANOVA results showed statistically differences ($p<0.05$), post hoc testing was performed for intergroup comparisons using Scheffe's test.

Results

1. Percentage yield of Mesona chinensis aqueous extract (MCAE)

All samples of *M. chinensis* explants were basically raw materials used in manufactures of black grass jelly in Thailand. They were from three major world exporters including China (2 sources, C1 and C2), Indonesia (N3) and Vietnam (V4). Regardless of the botanical origins, they possessed similar color (dark brown- black) and characteristic odor. However, their proportion of leaves and stems were considerably different. The majority of C1-explants were dried leaves, while C2-explants were mainly dried stems. N3-explants and V4-explants were dried stems with leaves.

Decoction is an extraction technique used especially for water-soluble and heat resistant components. In this experiment, all *M. chinensis* plants were subjected to 2 hours decoction with water (1:20 w/w) for three extraction cycles. Subsequently, the pooled aqueous extract was completely dried using freeze dryer. According to their botanical sources, the obtained *Mesona chinensis* aqueous extracts (MCAEs) were designated as MCAE-C1, MCAE-C2, MCAE-N3, and MCAE-V4, respectively. All MCAEs had similar typical appearance and sensory attributes. They were dark brown powder with herbal odor, mild astringency, and bitter taste. The range of % yield was 15.70%-18.95% (Table1).

Table 1 The percentage yield of *Mesona chinensis* aqueous extract (MCAE) from different botanical sources

Extract code	Botanical source	Yield (%w/w)
MCAE-C1	China (C1)	18.65
MCAE-C2	China (C2)	18.95
MCAE-N3	Indonesia (N3)	15.70
MCAE-V4	Vietnam (V4)	16.25

2. Bioactive compounds of MCAEs

Generally, phenolic compounds and flavonoids are the main natural bioactive compounds in plant extract. Therefore, total phenolic content (TPC) and total flavonoids content (TFC) of MCAEs obtained from different botanical sources were determined (Table 2). Folin-ciocalteu's and aluminum chloride colorimetric methods are usually applied to quantify phenolics and flavonoids in plant extract. The range of total phenolic contents (TPC) and total flavonoid contents (TFC) found in the MCAEs obtained from different botanical sources were 68.83 to 133.27 mg GAE/g extract and 3.49 to 5.83 mg QE/g extract, respectively.

Table 2 Total phenolic content (TPC) and total flavonoid content (TFC) of *Mesona chinensis* aqueous extract (MCAE) from different botanical sources

Extract code	Botanical source	Total phenolic content (mg GAE/ g extract)	Total flavonoid content (mg QE/ g extract)
MCAE-C1	China (C1)	68.63 ± 4.69^{C}	3.49 ± 0.46^{C}
MCAE-C2	China (C2)	133.27 ± 8.06^{A}	5.83 ± 0.38^{A}
MCAE-N3	Indonesia (N3)	112.32 ± 1.50^{B}	4.42 ± 0.22^{BC}
MCAE-V4	Vietnam (V4)	126.65 ± 5.46^{AB}	5.24 ± 0.68^{AB}

Data are expressed as mean ± SD (n=3).

$^{A-C}$ values within a column with different letters were significantly different at $p<0.05$

GAE = Gallic acid equivalent; QE = Quercetin equivalent.

3. Biological activities of MCAEs

In this research, the antioxidant activity and anti-hyperglycemia effect of MCAEs were evaluated using DPPH radical scavenging activity assay, and *in vitro* α-glucosidase inhibitory activity method, respectively. DPPH assay evaluated the ability of MCAE to eliminate free radicals ($DPPH^{\bullet}$) by donating a hydrogen atom. The scavenging effects of MCAEs were illustrated by the plots between the percentages scavenging versus the concentrations (Figure 1). The obtained results revealed the scavenging activity of MCAE was dose dependent relationship. At low MCAEs concentration (0-62.5 μg/mL), all graphs were linear with high coefficient of determination (R^2 = 0.9925-0.9979).

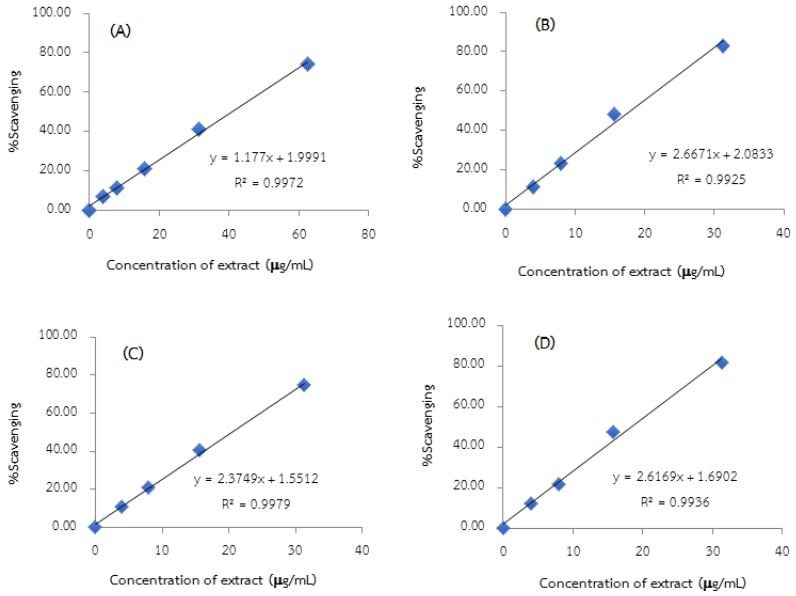

Figure 1 Correlations of the percentage DPPH radical scavenging and concentration of MCAEs from different botanical sources (A) MCAE-C1 from China, (B) MCAE-C2 from China, (C) MCAE-N3 from Indonesia, (D) MCAE-V4 from Vietnam

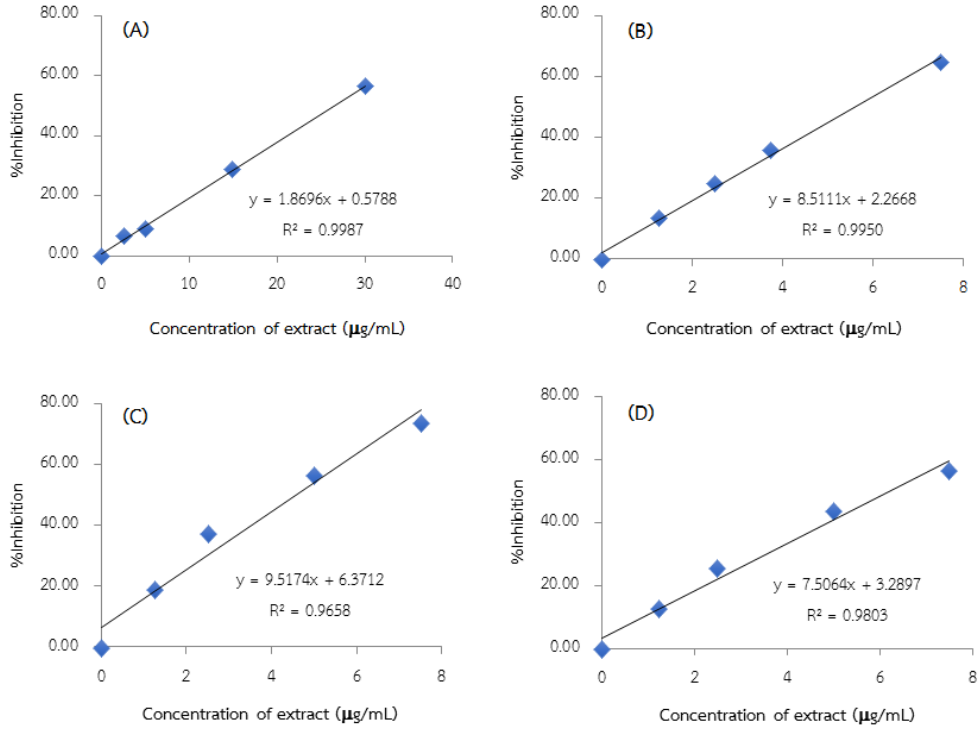

Figure 2 Correlations of the percentage inhibition of α-glucosidase and concentration of MCAEs from different botanical source (A) MCAE-C1 from China, (B) MCAE-C2 from China, (C) MCAE-N3 from Indonesia, (D) MCAE-V4 from Vietnam

The α-glucosidase inhibitory activities of MCAEs were determined using *p*NPG as substrate. Theoretically, α-glucosidase hydrolyzed *p*NPG into yellow-colored products (PNP) which has maximum absorbance at 405 nm. The enzyme activity is directly to the production rate of PNP. Inhibition effect of α-glucosidase by MCAEs at different concentrations were shown in Figure 2. Similar to DPPH scavenging results, at low MCAEs concentration (0-30 μg/mL), all MCAEs inhibited α-glucosidase in dose-dependent manner.

To compare the effectiveness of MCAEs from different botanical origins, the half maximal inhibitory concentrations or IC_{50} values of all MCAEs were then calculated (Table 3) from their corresponding linear equations (Figure 1 and Figure 2). As general rule, the lower IC_{50} value indicates the higher antioxidant capacity and more effective inhibition against α-glucosidase of MCAE.

Table 3 Antioxidant activity and α-glucosidase inhibitory activity of *Mesona chinensis* aqueous extract (MCAE) from different botanical sources

Extract code	Botanical source	IC_{50} value (μg/mL)	
		DPPH scavenging inhibitory activity	α-glucosidase inhibitory activity
MCAE-C1	China (C1)	37.98 ± 0.63^{A}	26.86 ± 0.40^{A}
MCAE-C2	China (C2)	17.54 ± 0.51^{C}	5.70 ± 0.23^{B}
MCAE-N3	Indonesia (N3)	21.31 ± 0.84^{B}	4.77 ± 0.17^{B}
MCAE-V4	Vietnam (V4)	18.54 ± 0.07^{C}	5.70 ± 0.48^{B}

Data are expressed as mean ± SD (n=3).

$^{A-C}$ values within a column with different letters were significantly different at $p<0.05$

4. Enzyme inhibition kinetics

Due to its potent inhibitor, MCAE-C2 was chosen for study inhibition kinetics against α-glucosidase. Michaelis-Menten plot and Lineweaver-Burk plot for MACE-C2 in the presence of *p*NPG at increasing concentrations were constructed to identify the type of inhibition. According to Michalis-Menten plot (Figure 3), the presence of MACE slowed down the reaction velocity and the decline rate was highly governed by MCAE concentration.

Additionally, it was clearly illustrated that at any concentration of MCAE-C2, the increase of substrate concentration did not liberate the inhibition. Furthermore, the Lineweaver-Burk plot (Figure 4) displayed a series of straight lines which intersected at the same point in the second quadrant. The increase of MCAE concentration caused the vertical axis intercept (1/*v*) to increase which lead to the decrease of V_{max}. The results suggested that type of inhibition was a noncompetitive mode (Binh *et al.*, 2016). In other words, MCAE, as an inhibitor, was not binding free enzyme at active sites (Zhang *et al.*, 2015). This kind of attachment would form an inactive enzyme–inhibitor complex (EI) and enzyme-substrate–inhibitor complex (ESI) which could not disconnect and not yield a product of reaction (Kim *et al.*, 2005).

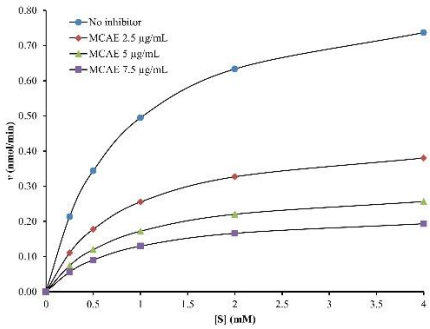

Figure 3 Michaelis-Menten plot for the kinetic analysis of α-glucosidase activity inhibited by *M. chinensis* aqueous extract; *v* is reaction velocity (nmol/min); [S] is concentration of *p*NPG (mM)

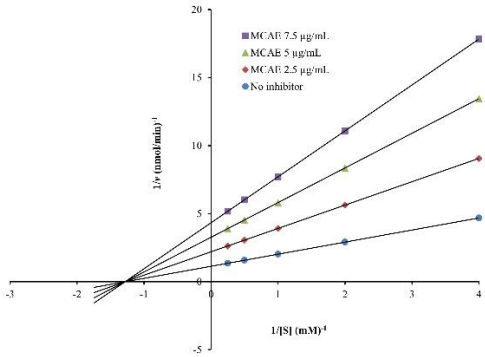

Figure 4 Lineweaver-Burk plot for the kinetic analysis of α-glucosidase activity inhibited by *M. chinensis* aqueous extract; 1/*v* is reciprocal of reaction velocity $(nmol/min)^{-1}$; 1/[S] is reciprocal of substrate concentration $(mM)^{-1}$

Discussion

1. Percentage yield of MCAEs

It is well acknowledged that bioactive compounds profiles of any medical plant are governed by several factors such as genetic, climate, topography, and field management. Therefore, effects of botanical sources of *M. chinensis* on biological properties of their aqueous extracts were studied to provide significant information for source selection.

In Thailand, black grass jelly companies individually imported dried *M. chinensis* plants from pre-selected botanical source to maintain its characteristic of black grass jelly, especially on textural quality. For conventional processing, dried *M. chinensis* plants are boiled in alkaline solution (1% Na_2CO_3) for six hours. The obtained filtrate is blended with starch solution to form black grass jelly. However, alkalinity and prolonged heat caused a significant decrease of antioxidative activity of extract of *M. chinensis* (Yen & Hung, 2000). Therefore, in this study we used water and maintained extraction temperature lower than 100 °C.

Based on % the extraction yield (Table1), China (18.65-18.95%) was the most promising botanical source for MCAE production than Vietnam (16.25 %) and Indonesia (15.70%). The average % yield of MCAEs from China explant was about 3% higher than the previous reported value (Yen *et al*., 2001**)**. The triple extraction cycle performed in this research and longer extraction time might resulted in a greater percentage yield.

2. Bioactive compounds of MCAEs.

Phenolic compounds and flavonoids have been reported to have many biological effects, especially on antioxidant and anti-hyperglycemia (Hung & Yen, 2002; Chusak *et al*., 2014; Sasmita *et al*., 2017; Lim *et al*., 2018). For instance, phenolic compounds identified in *M. chinensis* plant were protocatechuic acid, caffeic acid, syringic acid, vanillic acid and *p*-hydroxybenzoic acid (Yen *et al*., 2003).

As we expected, botanical source statistically regulated TPCs and TFCs of MCAEs ($p<0.5$). TPC ranged from 68.83 to 133.27 mg GAE/g extract increasing in the following order MCAE-C2 > MCAE-V4 > MCAE-N3> MCAE-C1 (Table 2). The average TPC value of all MCAEs (110.22 ± 29.07 mg GAE/g extract) was in the range of reported values from previous studies (33.33-238.7 mg GAE/g extract) (Yen *et al*., 2001; Jeng *et al*., 2013). Interestingly, TPC of MCAE-C1 was approximately 50% lower than those of other MCAEs. This difference may be from the majority of *M. chinensis* explants from C1 source were dried leaves. Nonetheless, there has been no previous research comparing bioactive compounds content in stems and leaves of *M. chinensis* explants. One of the major polysaccharide gums found in *M. chinensis* leaves was pectin (Tao *et al*., 2008). Pectin and other water-soluble compounds might be co-extracted with phenolic compounds. Therefore, it could be stated that *M. chinensis* explants contained majority of dried stems were more favorable sources for MACE production.

Unsurprisingly, all MCAEs contained a small number of flavonoids. Flavonoids contain two benzene rings connected by a heterocycle pyrene ring resulting in their poor solubility in water. Nonetheless, kaempferol and apigenin were identified in water extract of *M. Chinensis* (Yen *et al*., 2003). TFC ranged from 3.49 to 5.83 mg QE/g extract increasing in the following order, MCAE-C2 > MCAE-V4 > MCAE-N3> MCAE-C1, which was similar to TPC result (Table 2). TFC of MCAE-C2 was slightly higher than the reported value (4.05±0.06 mg QE/g extract) from previous study (Jeng *et al*., 2013).

3. Biological activities of MCAEs

For DPPH scavenging assay, IC_{50} of MACEs ranged from 17.54 to 37.98 μg/mL (Table 3). However, IC_{50} of MCAE-N3, MCAE-V4 and MCAE-C2 were about 2 times lower than that of MCAE-C1. These results were in good agreement with their total phenolic contents (Table 1). Thus, MCAE-C2 was the most potent antioxidant due to its lowest IC_{50} value (17.54 μg/mL). This IC_{50} value was in accordance with previous research done by Yen *et al.* (2003). In addition, the average IC_{50} value of all MCAEs was determined to be 23.84±9.56 μg/mL which was about 7 times higher than that of ascorbic acid (positive control). This relative potency of antioxidant was parallel with the finding done by Chusak *et al*. (2014).

Based on enzyme inhibition assay, IC_{50} of MACEs ranged from 4.77 to 26.86 μg/mL (Table 3). IC_{50} of MCAE-N3, MCAE-V4 and MCAE-C2 were about 5 times lower than that of MCAE-C1. These obtained results were in harmony with their total phenolic contents (Table 1). The correlation between total phenolic contents (X) and IC_{50} of α-glucosidase inhibitory activity of MCAEs(Y) was Y=-0.3475X+49.097(R^2=0.8859). Chusak *et al.* (2014) reported that polyphenolic compounds and flavonoids in water extract from *M. chinensis* contributed to its intestinal α-glucosidase inhibitory activities. It is well documented that phenolic compounds efficiently impede the activities of carbohydrate-hydrolyzing enzymes because of their ability to bind with protein (Zhang *et al.*, 2015). The main interactions between phenolics and α-glucosidase are hydrophobic interactions, hydrogen bonding and van der Waals interaction (Seetaloo *et al.*, 2019). The average IC_{50} values of all MCAEs and acarbose (positive control) were determined to be 10.76±0.32 μg/mL and 414±40.39 μg/mL, respectively. Thus, it could be concluded that MCAEs was the potent inhibitor against yeast α-glucosidase activity. However, as previously reported, IC_{50} value of *M. chinensis* water extract on rat intestinal sucrase and maltase were 1.30±0.43 mg/mL and 4.66±0.26 mg/mL, respectively (Chusak *et al.*, 2014). This discrepancy was mainly due to different origins of α-glucosidase used in the assay(Kim *et al.*, 2005; Seetaloo *et al.*, 2019).

4. Enzyme inhibition kinetics

The inhibition type of α-glucosidase activity by MCAE was a noncompetitive manner. The binding of the MCAE to the α-glucosidase at allosteric sites lowered its activity (lower V_{max}) but did not affect the binding of substrate (unchanged K_m). The kinetic parameters of α-glucosidase inhibition by MCAE-C2 were K_m =0.7811 mM and K_i =2.67 μg/mL. The low K_i indicated that the more preference formation of EI complex than ESI complex (Binh *et al.*, 2016).

Conclusion

The results of this study provided useful information of the source selection of *M. chinensis* explants for a future commercial *Mesona chinensis* aqueous extract (MCAE) production. Most of imported *M. chinensis* plant were considered as potential sources according to total phenolic content, antioxidant activity and α-glucosidase inhibitory activity of MCAEs. However, it is noteworthy to state that stem explant of *M. chinensis* maybe favorable than leaves explant. The results also revealed that total phenolic content of MCAE was in good relationship with its DPPH radical scavenging activity (R^2=0.9810) and α-glucosidase inhibitory activity (R^2=0.8859). Phenolic compounds in MCAE significantly play an important role for the inhibition α-glucosidase. The inhibition type of α-glucosidase activity by MCAE was a noncompetitive type with the inhibitory constant (K_i) of 2.67 μg/mL.

Acknowledgement

The authors are grateful to Faculty of Pharmacy, Mahidol University and Pattanapoonphol Company for financial supporting and providing necessary facilities.

Disclosure statement

The authors declared that they have no conflicts of interest.

Funding

This research project was supported by Mahidol University.

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