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Contributed Paper

Inhibitory Effects of Phenolic Compounds in *Ocimum sanctum* Extract on the α-Glucosidase Activity and the Formation of Advanced Glycation End-products

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ABSTRACT

Hyperglycemia causes increased protein glycation which plays an important role in the development of chronic diabetic complications. Therefore, the inhibition of protein glycation is an alternative approach in decreasing the complications of diabetic pateints. This study primarily evaluated the antiglycation effects of five Lamiaceae plants (*Ocimum basilicum*, *O. americanum*, *O. sanctum* (green), *O. sanctum* (purple) and *Metha cordifolia* opiz.) using bovine serum albumin (BSA)-methylglyoxal (MGO) and histone-MGO models. Among Lamiaceae plant extracts, the crude ethanolic extract of *O. sanctum* (purple) was the most active species against protein glycation in both model proteins. The crude ethanolic extract of *O. sanctum* (purple) was partially purified by partitioning the extract into ethyl acetate (EA) and aqueous fractions and then investigating these fractions for advanced glycation end-products (AGEs)under different inducer models. The results indicated that the EA fraction of *O. sanctum* (purple) possessed strong inhibitory activities against AGE formation in both extracellular and intracellular proteins, including the use of different inducers. Moreover, this fraction also displayed the potent inhibition activity against α-glucosidase. The results of the LC-MS analysis of the EA fraction of *O. sanctum* (purple) revealed four phenolic compounds which were methyl eugenol, rosmarinic acid, luteolin and apigenin. These phenolics might be the bioactive compounds in the EA fraction of *O. sanctum* (purple) that contributed to antiglycation activities and antidiabetic properties through the inhibition of α-glucosidase activity, except for methyl eugenol which only showed α-glucosidase inhibition.

Keywords: antiglycation, *Ocimum sanctum* (purple), α-glucosidase activity, luteolin, rosmarinic acid, apigenin

1. INTRODUCTION

Prolonged hyperglycemia plays an important role in the development of diabetic complications leading to morbidity and mortality in diabetic patients. Several acute

complications of diabetes can cause lipid and protein metabolic alterations in the body and more chronic irreversible complications [1-2], such as retinopathy, cataracts, atherosclerosis, neuropathy and aging[3-4]. The glycation of protein is one of the main primary mechanisms in the progression of diabetic complications. The early stage of glycation involves the amino groups of proteins and the aldehyde groups of reducing sugars to form the Schiff base that are subsequently rearranged to produce more stable Amadori products through the oxidative pathways. In the next stage, theAmadori products undergo numerous complex reactions (dehydration, oxidation and cyclisation) and produce several highly reactive carbonyl species (RCS), such as glyoxal, methylglyoxal (MGO) and 3-deoxy-glucosone. Finally, such reactive carbonyl compounds can rapidly react with the amino groups of both extracellular and intracellular proteins leading to the formation of a variety of fluorescent and nonfluorescent advanced glycationend-products (AGEs)[5-6]. The accelerated AGE accumulation on extracellular proteins, such as albumin and low-density lipoproteins (LDL), induces protein dysfunction and tissue damage that is conductive to the vital factors for atherosclerosis and vascular basal membrane atrophy and the elevation of the blood pressure[3,7]. Furthermore, AGEs also form on intracellular proteins, such as histone, which is a composition of the DNA structure that results in genetic instability. Sugars other than glucose are also important in inducing protein glycation. In particular, pentoses, such as D-ribose, can participate in protein glycation much more than glucose. The highly reactive carbonyl species (RCS), glyoxal and methylglyoxal, were also efficient inducers for AGE formation. These compounds are up to 20,000 times more reactive than glucose[8-9]. The elevated AGE formation leads to a speed up in diabetic complications and modifications of proteins, lipids and DNA[10-11].

Therefore, the inhibition of AGE formation is one of the therapeutic approaches in the prevention of diabetic complications. Currently, the strong AGE inhibitor is aminoguanidine (AG), a hydrazine derivative synthetic inhibitor that showed the effective suppression of diabetic complications. Unfortunately, it proved to be toxic in long-term administration [12-14]. Therefore, efforts have been made to search for other safe and effective inhibitors against protein glycation.

Another therapeutic approach for the prevention of diabetic complications is to decrease postprandial hyperglycemia by retarding the absorption of glucose. Inhibition of α-glucosidase, which is a carbohydrate-hydrolyzing enzyme in the small intestine, has been considered. Recent attention has been focused on the bioactive compounds derived from natural plants having both antiglycation and α-glucosidase inhibitory properties. Several phytochemical investigations of the inhibitory effects on AGE formation have been reported. Procyanidins from cinnamon was shown to work effectively against protein glycation[15]. Flavan-3-ols from green tea exhibited the ability to scavenge carbonyl compounds [16]. Rutin and its metabolites showed inhibitory potential for non-oxidative AGE generation *via* BSA-glycation[4]. Numerous phenolic compounds from the Lamiaceae family have been isolated and identified as being physiologicaly active natural compounds. *Ocimum sanctum*, a popular culinary plant of the Lamiaceae family, is considered one of the better known medicinal plants in Thailand. Its leaf has been traditionally used to treat high blood pressure and to lower cholesterol. However, its bioactive constituents, which are thought to be beneficial in antidiabtic treatment and the prevention of diabetic complications, are little known. In the present study, the 5 plants in Lamiaceae family (*Ocimum sanctum* (purple), *Ocimum sanctum* (green), *Ocimum basilicum, Ocimum americanum*

and *Metha cordifolia* opiz.) which widely cultivate and consume in Thailand were screened for antiglycation activities. Moreover, the active compoundsfrom the *Ocimum sanctum* (purple) fraction, which are responsible for the anti-AGE formation in different model systems, were evaluated. In addition, we also investigated the inhibition mode of α-glucosidase of *Ocimum sanctum* (purple) fractions by comparing them with the standard phenolic compounds.

2. MATERIALS AND METHODS

2.1 Chemicals

Hexane, ethanol, sodium carbonate, sodium azide, D-glucose and D-ribose of analytical grade were used in this study. Methanol and acetronitrile were of HPLC grade. Rosmarinic acid (RA), luteolin (LU), apigenin (AP), aminoguanidine (AG), bovine serum albumin (BSA), methylglyoxal 30% solution, fetal calf thymus histones (type II S) were purchased from Sigma & Aldrich Company, USA.

2.2 Sample Preparation

Five species of plants in the Lamiaceae family used in this study were *O. sanctum* (purple), *O. sanctum* (green),*O. basilicum, O. americanum* and *M. Cordifolia* opiz.. Dried plant material (30g) was pre-extracted (x3 times) overnight with 300 mL of hexane at room temperature. Following filtration, the residue was extracted overnight with 80% ethanol (x3 times) at room temperature. Subsequent to filtration, the ethanolic supernatant was pooled and evaporated by rotary evaporator and then lyophilized as powder. The ethanolic powder was used for the primary investigation of antiglycation actvities.

The dried ethanolic powder of *O. sancum* (purple) (10g) was suspended in 30 mL of distilled water and then partitioned with ethyl acetate (EA) (50 mL x 3) to obtain the EA layer and the remaining aqueous layer. Both EA and aqueous layers were concentrated by the evaporator and then lyophilized. All fractions obtained from *O. sancum* (purple) were used for the antiglycation assay and the active fraction was selected for the purposes of identifying the bioactive compounds.

2.3 Antiglycation Activities

2.3.1 Antigylcation assay in BSA-MGO model

The evaluation for the inhibition of the middle stage of the protein was performed according to Peng[17]. Thirty microliters of 500 mM methylglyoxal (MGO) were mixed with 300 μL of 10 mg/mL BSA in the presence of 0.2 g/L of NaN_3. The BSA-MGO reaction mixture was incubated at 37°C for 3 days with and without the plant extracts, while the aminoguanidine (AG) was used as a positive control. The reaction solution was incubated at 37°C for 7days before AGE fluorescent measurement. The fluorescence intensity was measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm with a luminescence microplate reader (Synergy H4, Biotek). The percentage of the AGE inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = [(F_0 - F_t)/F_0] \times 100$$

Where F_t *and* F_0 represent the fluorescence intensity of the sample and the control, respectively. Different extract concentrations (50-500 μg/mL) providing 50% AGE inhibition (IC_{50}) were calculated from the graph of AGE inhibition (%) of various concentrations of the extract.

2.3.2 Antiglycation assay in histone-MGO model

According to a slightly modified method of Gugliucci et al. [18], histone was dissolved in 10 mM PBS buffer, pH 7.4 containing 150 mM NaCl and 0.01% NaN_3 to a final protein concentration of 1 mg/mL. It was incubated with methylglyoxal (MGO) to a final concentration of 1 mM. The histone-MGO reaction mixture was incubated with different concentrations

of plant extracts (50-500 μ/mL)at 37°C for 4 days. After incubation, AGE fluorescence intensity was determined using a luminescence microplate reader at an excitation wavelength of 370 nm and an emission wavelength of 440 nm.The percentage of the AGE inhibition was calculated using the same equation as in the BSA-MGO model.

2.3.3 Antiglycation assay in different reducing sugars

The protein model of BSA was incubated with different reducing sugars (0.5 M of D-glucose, D-ribose and MGO) at 37 °C in thepresence or absence of the EA and the aqueous fraction of *O. sanctum* (purple) for 7 days (D-ribose and MGO) and 14 days (D-glucose). The AGE fluorescence intensity was also measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm.

2.4 Identification of the Phenolic Compounds by LC-MS

The phenolic compounds in the active fraction of *O. sanctum* (purple) were identified using LC-MS analysis. The LC-MS system involved the Agilent 1100 series system, which consisted of degasser, a quaternary pump, an autosampler, a heating exchanger and diode array detector, as well as a MS detector, with Zorbax SB-C_{18} column (250x4.6 mm, particle size of 5 μM). The negative ion mode for mass spectra was adopted to analyze the phenolic compounds based on their ionization tendency. The phenolic compounds were eluted with a gradient mobile phase composed of 1% acetic acid in water (solvent A) and 1% acetic acid in methanol (Solvent B) at the flow rate of 0.3 ml/min. The gradient elution was performed as follows: 0-5 min, 10-22%B; 6-25 min, 22-50% B; 26-45 min, 50-95%B; 46-55 min, 95% B; 56-60 min, 95-10% B. and maintained at 95-10% B; 61-66min. The wavelength of the UV-Vis detector was set at 280 nm. Ions were scanned from 200 to 800m/z. The mass spectrum was compared with the mass spectrum of standards and has been described in previously published data.

2.5 Preparation of α-glucosidase (AGH) Solution from Rat Intestinal Acetone Powder

AGH solution was prepared using the procedure of Oki [19]. Firstly, 100 mg of intestinal acetone powder (Sigma Aldrich, Singapore) was mixed with 3 mL of 0.9% (w/v) sodium chloride solution and the solution was homogenized with a vortex for 30 s. The enzyme mixture was centrifuged at 10,000 rpm for 30 min at 4 °C. Finally, the AGH supernatant was kept in an ice bath and the α-glucosidase inhibitory activity assaywas recorded.

2.6 Assay of α-glucosidase Inhibitory Activity

The α-glucosidase inhibitory assay was performed using the slightly modified method of You[20].*p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) was used as the substrate**.** 70 μL of AGH supernatant (0.6 units/mL) was pre-incubated with 30 μL of the plant extract at various concentrations in a 96-well plate for 15 min at 37°C. After incubation, 100 μL of 0.5mM *p*NPG solution in 100 mM phosphate buffer pH 6.8 were combined and incubated for 15 min at 37°C under shaking conditions. The concentration of *p*NPG was hydrolysed by α-glucosidase to release *p*-nitrophenol (yellow reagent) that could be measured at 405 nmevery 10 min for 60 min by a micro plate reader (BioTek ELX808, UK). The kinetic inhibition study of α-glucosidase of the EA and aqueous fraction from *O. sanctum* (purple) at different concentrations was conducted. The ranges of final *p*NPG substrate concentration were 0.5, 1.0, 2.0and 3.0 0 mM for α-glucosidase inhibition assay. The types of inhibition, *Km* and *V max* values, were determined graphically from double-reciprocal Lineweaver-Burk plots $1/s$ versus $1/v$. The K_m is the Michaelis

constant and the V_{max} is the maximam rate of the enzymatic reaction. Meanwhile, the K_i value of the sample was obtained from the least square regression lines of the plots according to the reciprocal measurements of the fraction concentration versus the reciprocal measurements of the rate of the reactions, for which the formula is listed below. Acarbose and the standard phenolic compounds of methyleugenol, rosmarinic acid, luteolin and apigenin were used as standard inhibitors to be compared with the plant extracts

The Lineweaver-Burke equation for competitive inhibition type is:

$$\frac{1}{V} = \frac{K_m[I]}{V_{max}K_i[S]} + \frac{1}{V_{max}}\left(1+\frac{K_m}{[S]}\right)$$

The Lineweaver-Burke equation for mixed non-competitive inhibition type is:

$$\frac{1}{V} = \frac{K_m.1}{V_{max}[S]}\left(1+\frac{[I]}{Ki}\right) + \frac{1}{V_{max}}\left(1+\frac{[I]}{aKi}\right)$$

aK_i was obtained from :

$$\text{y-intercept} = \frac{1}{Vmax.aKi} \cdot \frac{1}{[I]} + \frac{1}{Vmax}$$

IC_{50} value was obtained from:

$$IC_{50} = K_i\left(1+\frac{[S]}{K_m}\right)$$

Where the K_m is the Michaelis constant and the K_i is the dissociation constant. The V_{max} is the maximam rate of the enzymatic reaction. The [S] represents the concentration of the pNPG substrate and [*I*] is the concentration of the inhibitor (the plant extracts or the standard phenolic compounds). The IC_{50} value is the concentration of the inhibitor providing 50% inhibitory activity.

2.7 Total Phenolic Content was Determined by Folin-ciocalteu Assay

The total phenolic content of the fraction was assessed by the Folin-Ciocalteu method with some modifications [21] and gallic acid was used as the standard phenolic compound. The extract which was redissolved in ethanol (250 µL) was transferred to a test tube containing 2.5 mL of distilled water. The sample was mixed with 500 µL of the Folin-Ciocalteu reagent and left to react for 5 min. The reaction mixture was neutralized by the addition of 500 µL of 20% (w/v) sodium carbonate (Na_2CO_3) and incubated for 1 h at room temperature. The absorbance was then measured at 765 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g sample.

2.8 Qualitative and Quantitative Analysis of Phenolic Compounds

The extracts were measured using the HPLC technique [23]. The column was a hypersil ODS, 5µM C_{18} (250 x 4.6 mm ID). The flow rate was set to 0.9 mL/min. The fingerprint profiles were recorded at an optimized wavelength of 280 nm. The mobile phase was 0.2% formic acid in water (solvent A) and 0.2% formic acid in acetonitrile (solvent B). The gradient elution was performed as follows: 0-5 min, 10% B; 5-25 min, 40% B; 26-31 min, 55% B; 32-40 min, 65% B; 41-55 min, 75% B. The injection volume was 10 µL (1mg/mL). The quantification of methyl eugenol, rosmarinic acid, luteolin and apigenin was determined based on peak area measurements.

2.9 Statistical Analysis

All triplicated experimental results were presented as means ± SD. One-way analysis of variance (ANOVA) was applied for comparison of the mean values. P value < 0.05 was regarded as significant. All statistical analyses were performed using SPSS software (SPSS 17.0 for windows; SPSS Inc., Chicago).

3. RESULTS AND DISCUSSION

The BSA-MGO and histone-MGO models used in our study have provided useful tools

for a primary investigation of the inhibitory effects of Lamiaceae plants on the protein glycation in both extracellular and intracellular proteins.Histone, containing a high percentage of arginine and lysine residues, was selected as a target of intracellular protein glycation, while BSA plasma protein was a target of extracellular protein glycation. Methylglyoxal (MGO) was used as an inducer for AGE formation due to its efficiency. After a week of incubation time, the formation of AGE fluorescent was measured an excitation wavelength of 370 nm and an emission wavelength of 440 nm using a luminescence microplate reader. Table 1 shows the inhibitory effects of the ethanolic extracts from five Lamiaceae plant extracts on the AGE formation in both BSA-MGO and histone-MGO. Among the ethanolic extracts, the *O. sanctum* (purple) extract demonstrated the strongest inhibitory effect against AGE formation in both BSA-MGO and histone-MGO models when compared with the other extracts,with IC_{50} values of 0.402 mg/mL and 0.645 mg/mL, respectively.However, the IC_{50}value of these extracts were less effective than aminoguanidine (AG), which is an antiglycative standard inhibitor in both model proteins (IC_{50} values of 0.092 and 0.074 mg/mL).

Ethyl acetate (EA) and aqueous fractions obtained from the partial purification process using solvent partitioning of the ethanolic extract of *O. sanctum* (purple) were also used to assess the antiglycation activity in BSA-MGO and histone-MGO models. The EA fraction of *O. sanctum* (purple) showed the strong suppressive effect against AGE formationin both BSA-MGO and histone-MGO models with IC_{50} values of 0.294 and 0.290 mg/mL, while its aqueous fraction showed a weak inhibitory effect with IC_{50} values of >1 and 0.804 mg/mL, respectively.A similar finding was reported by Hussain *et al.* [22] who revealed that the ethyl acetate fraction of *Phlomis bracteosa* has a stronger antiglycation effect than the other fractions.

Due to the efficiency in suppressing the AGE formation in both extracellalar and intracellular models of the EA fraction, the bioactive

Table 1. Antiglycation activities in extracellular and intracellular model proteins of Lamiaceae plants.

Extract	Antiglycation activity	
	IC_{50} (mg/mL)	
	BSA-MGO	**Histone-MGO**
Ethanolic extract		
O. basilicum	0.850 ± 0.0	1.02 ± 0.1
O. americanum	0.700 ± 0.0	>2.00
O. sanctum (green)	0.769 ± 0.1	1.11 ± 0.1
*M. cordifolia*opiz.	1.10 ± 0.6	0.918 ± 0.1
Aminoguanidine	0.092 ± 0.0	0.074±0.0
O. sanctum (purple)	0.402 ± 0.0	0.645 ± 0.0
- EA fraction	0.294 ± 0.0	0.290 ± 0.0
- Aqueous fraction	>1	0.804 ± 0.0

- EA fraction is ethyl actetate fraction

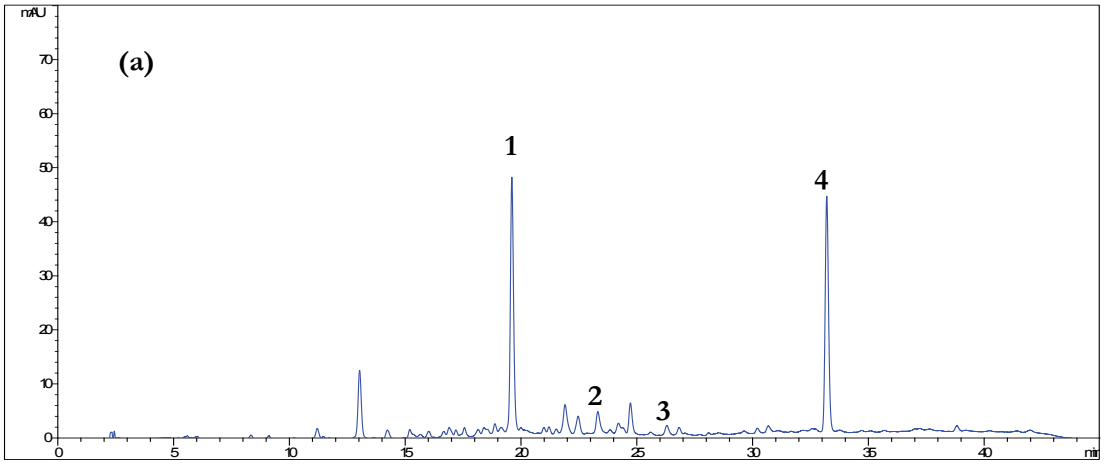

Figure 1. (a) HPLC chromatogram at 280 nm of ethyl acetate fraction from O. *sanctum* (purple); **(b)** Mass spectrometry chromatogram of O. *sanctum* (purple) extract: (1) *m/z* 359.0, (2) *m/z* 285.0, (3) *m/z* 269.1 and (4) *m/z* 178.1, respectively; **(c)** Chemical structure of active compounds: (1) rosmarinic acid, (2) luteolin, (3) apigenin and (4) methyl eugenol, respectively.

compounds in this fraction, particularly the phenolic compounds that might be responsible for AGE inhibition, were identified. The HPLC chromatogram of the EA fraction of *O. sanctum* (purple) are shown in Figure1 (a). According to a comparison using the mass spectrum (MS) of standards, the peaks in the chromatogram of the EA fraction were identified as rosmarinic acid (peak 1, m/z 359.0), luteolin (peak 2, m/z 285.0), apigenin (peak 3, m/z 269.1) and methyl eugenol (peak 4, m/z 178.1)(Figure 1(b))and were then compared with the mass spectrum standards. The negative mode ESI-MS is effective for these compounds. Rosmarinic acid, luteolin, apigenin and methyl eugenol provided the deprotonated molecule at 359.0, 285.0, 269.1 and 178.1 $[M-H]^-$ that confirmed the presence of these compounds in the EA fraction of *O. sanctum* (purple). Furthermore, the amounts of rosmarinic acid (RA), methyl

Table 2. Inhibitory effects of *O. sanctum* (purple) extracts on AGE formation in Bovine serum albumin (BSA) induced by glucose, ribose and methylglyoxal (MGO).

Extract	IC_{50} values (mg/mL)		
	glucose-BSA	ribose-BSA	MGO-BSA
EA extract	0.432±0.01[a]	0.434±0.03[b]	0.294±0.02[b]
aqueous extract	>1	0.825±0.02	>1
Standards	IC_{50} values (mg/mL)		
	glucose-BSA	ribose-BSA	MGO-BSA
Luteolin	0.006±0.00[a]	0.024±0.00[c]	0.017±0.00[b]
Apigenin	0.012±0.00[a]	0.032±0.00[b]	0.016±0.00[a]
rosmarinic acid	>0.2	0.164±0.02[b]	0.104±0.01[a]
aminoguanidine(AG)	0.066±0.00[a]	0.152±0.01[c]	0.092±0.00[b]
Methyl eugenol	NI	NI	NI

-Values are expressed as means±SD.

- [a-c] Means in the row followed by different letters are significantly different ($P<0.05$)

- NI is not inhibition

Table 3. Total phenolic content and total content of the EA and aqueous fractions of *O. sanctum* (purple) compared with the standard phenolic compounds.

Extract	Total phenolic content (mg GAE/g)	Total content (mg/g extract)			
		RA	ME	LU	AP
EA extract	17.06±0.0	6.49	2.82	0.97	0.41
aqueous extract	0.88±0.0	ND	ND	ND	ND

GAE; gallic acid equivalent, RA; rosmarinic acid, ME; methyleugenol, LU; luteolin and AP; apigenin, ND; not detected

eugenol (ME), luteolin (LU) and apigenin (AP) in the EA fraction were measured using HPLC analysis. The results showed that the EA fraction contained high amounts of rosmarinic acid (6.49 mg/g extract), followed by methyl eugenol (2.82 mg/g), luteolin (0.97 mg/g extract) and apigenin(0.41 mg/g extract), respectively, while these phenolic compounds were not detected in the aqueous fraction (Table 3).This result is in agreement with previous studies which have reported that rosmarinic acid, luteolin and luteolin glycosides were generally found in the Lamiaceae plant family[23-25].

It's well understood that the advanced glycation end-products (AGEs) are formed between proteins and by reducing sugars through oxidative and non-oxidative pathways. Therefore, the inhibitory effects of EA and the aqueous fractions from *O. sanctum* (purple) on the glycation of BSA induced by different reducing sugars (glucose, ribose and MGO) were investigated and compared with the selected standard phenolic compounds (luteolin, apigenin, romarinic acid and methyl eugenol). Table 2 indicatesa strong inhibitory effect of the EA fraction from *O. sanctum* (purple) against the glycation of BSA induced by MGO (IC_{50} value of 0.294 mg/mL), glucose (IC_{50} value of 0.432 mg/mL)and ribose (IC_{50} value of 0.434 mg/mL). However, its aqueous fraction exhibited weak inhibitory effects in 3 different inducer models. These results imply that the active compounds in the EA fraction of *O. sanctum* (purple) displayed a potent suppressing ability on the AGE formation that was not only induced by MGO and ribose, but also by ribose and glucose. In the case of the selected phenolic standards including luteolin, apigenin and rosmarinic acid, these standard compounds also demonstrated the strong AGE suppressive capabilities induced by glucose (IC_{50} value of 0.006 to 0.2 mg/mL), ribose (IC_{50} value of 0.024 to 0.164 mg/mL) and methylglyoxal (IC_{50} value of 0.016 to 0.104 mg/mL). However, methyl eugenol showed no antiglycation activities with all different inducers. It was suggested that some of the phenolic compounds (luteolin, apigenin and rosmarinic acid) in the EA fraction of *O. sanctum* (purple) might be responsible for AGE inhibition.

Alpha-glucosidase is a key enzyme involved in sugar metabolism. The α-glucosidase inhibitionis considered to be one of several therapeutic approaches used to decrease the

Table 4. Results of calculated *Ki* and IC_{50} values of α-glucosidase inhibitory activity and type of inhibition of the EA and aqueous fractions of *O. sanctum* (purple) compared with the standard phenolic compounds.

Extract	α-glucosidase inhibition		Type of inhibition
	***Ki* (mg/mL)**	**IC_{50}(mg/mL)**	
EA extract	1.02	1.72	Mixed
aqueous extract	3.82	8.02	Mixed
Standards	***Ki* (mg/mL)**	**IC_{50}(mg/mL)**	**Type of inhibition**
luteolin	0.09	0.14	Mixed
apigenin	0.14	0.24	Mixed
rosmarinic acid	1.21	2.13	competitive
methyl eugenol	2.04	2.80	competitive
acarbose	0.10	0.19	Mixed

hyperglycemia in diabetic patients. In this study, the inhibitory effects of the EA and aqueous fractions of *O. sanctum* (purple)on α-glucosidase were also determined by being compared with the standard phenolic compounds, from which the dissociation constant (*Ki*) and the IC_{50} value were also determined. Table 4 presents the results of *Ki* and IC_{50} values for the α-glucosidase inhibitory activity of the EA and aqueous fractions from *O. sanctum* (purple) compared with the selected standard phenolic compounds and acarbose. The EA fraction from *O. sanctum* (purple)showed strong inhibitory activity with IC_{50} and *Ki* values of 1.72 and 1.02 mg/mL, respectively, while its aqueous fraction revealed weak levels of inhibition (IC_{50} and *Ki* values of 8.02 and 3.82 mg/mL, respectively). The strong inhibitory activity of the EA fraction could be related with its high content of phenolic compounds which are listed in Table 3. However, the EA fraction was less effective than acarbose which is the synthetic standard inhibitor (IC_{50} and *Ki* values of 0.19 and 0.12 mg/mL, respectively). In the case of the selected phenolic compounds, luteolin and apigenin showed strong inhibitory activity in terms of their IC_{50} value (0.14 and 0.25 mg/mL, respectively) and their *K*i values (0.09 and 0.15 mg/mL, respectively). On the other hand, rosmarinic acid and methyl eugenol showed a weak inhibition against the α-glucosidase with IC_{50} values of 2.13 and 2.80 mg/mL, respectively. It was suggested that the strong α-glucosidase inhibitory activity of the EA fraction could be attributed to the phenolic compounds, particularly flavonoids luteolin and apigenin[20], [26-27].

Moreover, the types of α-glucosidase inhibition of the EA and aqueous fractions of *O. sanctum* (purple) were also determined by being compared with the selected standard phenolic compounds. The Lineweaver-Burk plot of their inhibition is displayed in Figure 2. Both EA and aqueous fractions of *O. sanctum* (purple) generated straight lines which had different intersections on the *x*-axis, indicating that their α-glucosidase inhibition were of the mixed non-competitive type. The mixed non-competitive inhibition of these fractions indicated that their inhibition resulted from the binding of both the free enzymes or in the enzyme-substrate complex. In the case of the standard phenolic compounds, both luteolin and apigenin also showed their inhibition types as being of the mixed non-competitive inhibitor type. While, romarinic acid and methyl eugenol displayed the patterns of competitive inhibition due to their intersections on the *y*-axis at the same point, indicating that both of these compounds compete with the substrate in binding to the active site of the enzyme. Similar results were reported by Tadera[28] and Lin[29], who demonstrated that the inhibition of luteolin on yeast α-glucosidase was a mixed-typed inhibitor, while that of rosmarinic acid was a competitive inhibitor. These results may also suggest that these phenolics (rosmarinic acid, methyl eugenol, luteolin and apigenin) might be the bioactive compoundsin the EA fraction of *O. sanctum* (purple) which contributed to the α-glucosidase inhibitory activity. These results are very encouraging and could lead to the development of therapeutic approaches used to treat diabetic patients.

4. CONCLUSIONS

This study investigated the inhibitory effects of five Lamiaceae plants on the formation of advanced glycation end-products (AGEs) in both extracellular and intracellular model proteins. The most active extract was found in the ethanolic extract of *O. sanctum* (purple). After of *O. sanctum* (purple) was partially purified by partitioning method, its ethyl acetate fraction showed the strong inhibitory activities against AGE formation induced by different inducers and also displayed high α-glucosidase inhibition based on the mixed non-competitive inhibition

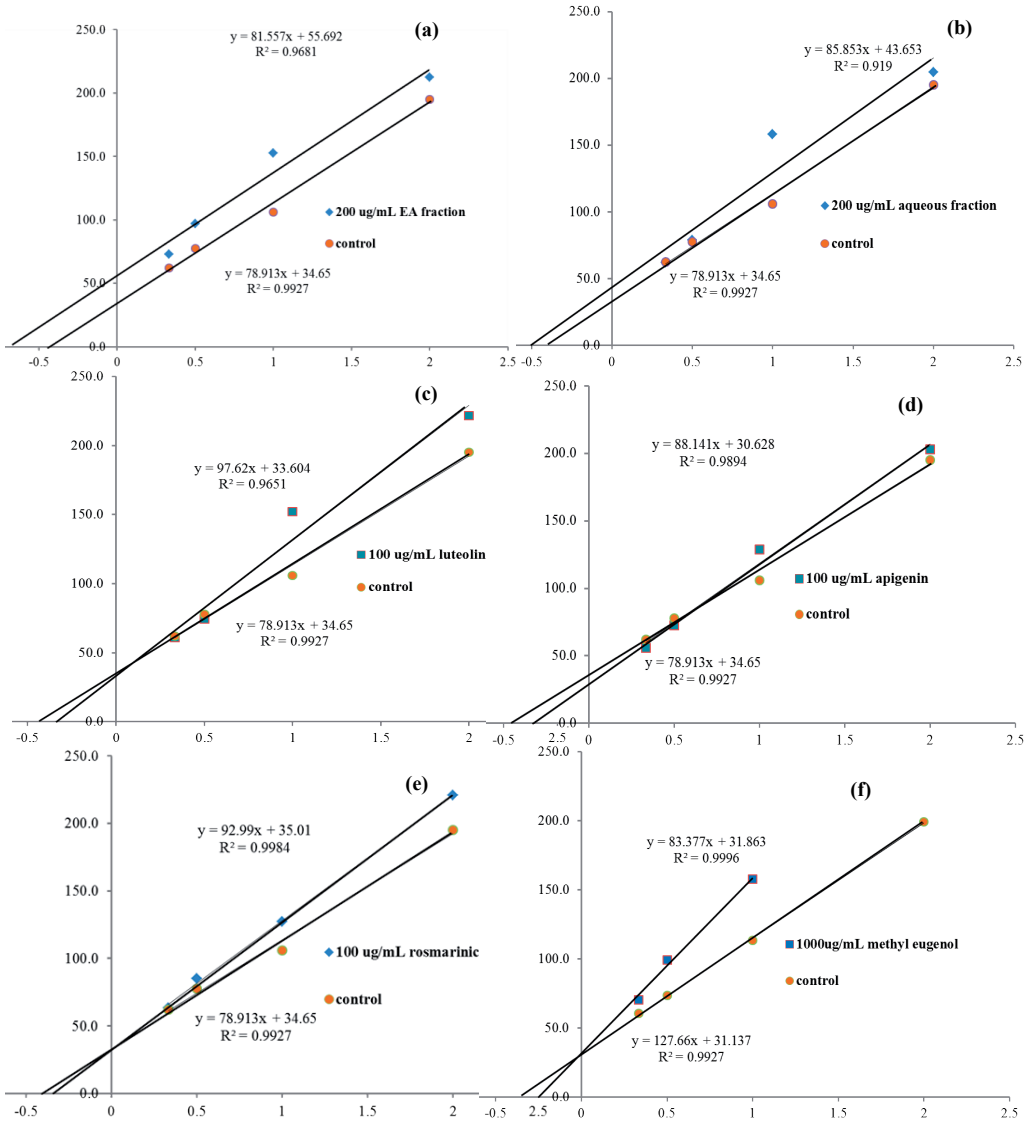

Figure2. The Linweaver-Burk plots of the EA (a) and aqueous (b) fractions of *O. sanctum* (purple) at different concentrations (0 and 200 µg/mL). Notably, (c), (d)and (e) were luteolin, apigenin and rosmarinic acid (at 0and 100 µg/mL), repectively, and (f) was methyl eugenol (at 0 and 1 mg/mL).

mode. According to the HPLC chromatogram, the EA fraction was identified by LC-MS technique as rosmarinic acid, methyl eugenol, luteolin and apigenin. The results implied that the strong AGE inhibition and α-glucosidase inhibition of the EA fraction of *O. sanctum* (purple) might be ascribed to some of the phenolic compounds (luteolin, apigenin and rosmarinic acid). On the basis of these results, these bioactive compounds might potentially be alternative resources for therapeutic adjuncts for the prevention and inhibition of diabetic complications.

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