Inhibitory Effects of Lamiaceae Plants on the Formation of Advanced Glycation Endproducts (AGEs) in Model Proteins

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***Abstract*—Protein glycation and oxidative stress caused by chronic hyperglycemia play vital role in diabetic complications. This study focused on the evaluation of the antiglycation effect of four Lamiaceae plants (*Ocimum sanctum*, *O. basilicum*, *O. americanum* and *Metha cordifolia* opiz.). Among the ethanolic extracts, *O. sanctum* extract exhibited high content of phenolic compounds and strong antioxidant activity. Chemical composition analyzed by HPLC revealing two major phenolic conpounds in *O. sanctum* extracts as rosmarinic acid (4.43 mg/g) and luteolin (0.96 mg/g). In the antiglycation assays, bovine serum albumin (BSA) and histone which were used as model proteins for investigation in the presence of methylglyoxal (MGO) with or without the extracts comparing with the authentic phenolic compounds. The results showed that *O. sanctum* extract possessed a potent antiglycation activity in both BSA and histone models with 23.4% and 43.0 % inhibition at the concentration of 500 and 250 μg/mL, respectively. The results indicated that *O. sanctum* which contained high phenolic compounds has potential to prevent protein glycation caused by oxidative stress.**

***Index Terms*—protein glycation, diabetic complications, lamiaceae plants, bovine serum albumin, histone**

I. Introduction

Nowadays, the main cause of both morbidity and mortality in diabetic patients results from the diabetic complication [1]. The prolonged hyperglycemia plays important role in the development of chronic diabetic complications leading to change of multiple cells *in vivo*. Besides, the effect of hyperglycemia facilitates several mechanisms, such as increased oxidative stress and nonenzymatic protein glycation [2], [3]. The nonenzymatic protein glycation is the reaction between the carbonyl group of reducing sugars with the amino group of proteins leading to the formation of advanced glycation endproducts (AGEs). AGEs can cross-link and accumulate with various proteins in body tissue.

The accelerated AGE accumulation *in vivo* induces protein dysfunction and damages in all tissues resulting in several diabetic complications and age-related degeneration. For example, the oxidative glycation of low-density lipoproteins (LDL) is initiative factor of atherosclerosis [4].

Therefore, the inhibition of AGEs formation is one of the therapeutic approaches for the prevention of diabetic complications. Currently, there are many synthetic AGE inhibitors that showed effectiveness against AGEs formation and breaking cross-linked proteins *in vivo*, such as aminoganidine [5]. However, their practical applications are limited because of their toxicity and severe side effects. In this regard, the effort has been made to find the natural compounds from herbs against protein glycation. The genus *Ocimum* and *Metha* under Lamiaceae family are the popular culinary plants that are considered as medicinal herbs in Thailand. Their leaves have been traditionally used to treat a variety of symptoms, including skin diseases, gastric and hepatitis disorders and kidney malfunction [6]. Moreover, they have been widely used for treating high blood pressure and lowering cholesterol. In present days, Lamiaceae plants were widely studied as natural antioxidant resources due to their high contents of phenolic compounds [7]. Numerous phytochemical investigations of *Ocimum* plants have shown that terpenoids and phenolic derivatives are the main components of these plants. These compounds have been reported on the potential of their antibacterial, antifungal, antioxidant, anti-inflammatory and radio-protective activities [8], [9]. However, bioactive constituents effective against protein glycation have not been investigated in detail. In this study, the four plants from Lamiaceae family (*O. sanctum*, *O. basilicum*, *O. americanum* and *M. cordifolia* opiz.) were primarily investigated for their total phenolic contents and antioxidant activity. The chemical compositions of the ethanolic extract from these plants were also analyzed by HPLC technique. Furthermore, their inhibitory effects on AGEs formation in the extracellular (bovine serum albumin) and intracellular (histone) proteins were evaluated in comparison with some authentic phenolic compounds.

II. Materials and Methods

A. Chemicals

Hexane, ethanol, sodium carbonate, sodium azide, formic acid were analytical grade. Methanol, acetronitrile

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were HPLC grade. Folin-Ciocalteu reagent, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, gallic acid, rosmarinic acid, luteolin, apigenin, aminoguanidine (AG), bovine serum albumin (BSA), methylglyoxal (MGO) 30% solution, fetal calf thymus histones (type II S) were purchased from Sigma & Aldrich Company, USA.

B. Preparation of Ethanolic Extracts

Four species of plants in Lamiaceae family used in this study were *O. sanctum, 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. After filtration, the residue was extracted overnight with 80% ethanol (x3 times) at room temperature. After filtration, the ethanolic supernatant was pooled and evaporated by rotary evaporator, and then lyophilized as powder. The ethanolic extract was used for further study.

C. Total Phenolic Content Was Determined by Folin-Ciocalteu Assay

The total phenolic content of each extract was assessed by the Folin-Ciocalteu method with some modifications [10] and gallic acid was used as the standard phenolic compound. The extract which 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.

D. In Vitro Determination of Antioxidant Activity by Using DPPH Radical Scavenging Activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of different sample extracts was determined [11]. 100 μL of DPPH radical solution (0.1 mM $DPPH^{\bullet}$ in methanol) was well mixed with 300 μL of the extract and incubated for 30 min at room temperature. The decrease in absorbance caused by the proton donating property of the active compounds was measured at 517 nm with microplate reader Biotek ELX808. The percent DPPH radical scavenging activity was calculated using the following formula:

$$DPPH\ radical\ scavenging\ effect(\%) = [(A_o\text{-}A_1)/A_o] \times 100$$

where A_o represents the absorbance of the control solution and A_1 represents the absorbance of the extract solutions.

E. Qualitative and Quantitative Analysis of Phenolic Compounds

The extracts were measured using HPLC technique. The column was a hypersil ODS, 5 μM 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 acids 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%; 32-40 min, 65% B; 41-55 min, 75% B. The injection volume was 10 μL (1mg/mL). The quantification of each compound was determined based on peak area measurements.

F. Antiglycation Activities

1) Antigylcation assay in BSA-MGO model

The evaluation for the inhibition of the middle stage of protein was slightly modified according to Peng [5]. 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℃ for 4 days with and without the plant extracts of 500 μg/mL. Aminoguanidine (AG) was used as a positive control. The fluorescence intensity was measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm with a Perkin Elmer LS-50B. The percentage of the AGE inhibition was calculated using the following equation:

$$Inhibition\ (\%) = [(F_0 - F_t)/F] \times 100$$

where F_t *and* F_0 respectively represent the fluorescence intensity of the sample and the control of mixtures.

2) Antiglycation assay in histone-MGO model

According to a slightly modified method of Gugliucci [12], 100 μL of 10 mg/mL histone in 10 mM PBS buffer pH 7.4 containing 150 mM NaCl and 0.01% Na_2N were incubated with 100 μL of 10 mM methylglyoxal (MGO). The histone-MGO reaction mixture was incubated at 37℃ for 4 days with the plant extracts of 250 μg/mL. After incubation, AGE fluorescence spectra was determined at an excitation wavelength 370 nm and an emission wavelength 440 nm with a Perkin Elmer LS-50B. The percentage of the AGE inhibition was calculated using the same equation as in the BSA-MGO model.

G. Statistical Analysis

All experimental results were presented as means ± SD in triplicate. 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).

III. Results and Discussions

A. Chemical Compositions of Lamiaceae Plants

The total phenolic contents in ethanolic extract of four species from Lamiaceae plants were determined by the Folin-Ciocalteu method and expressed as mg gallic acid equivalent (GAE) per g of dried sample. Table I shows their phenolic contents ranging from 31.5 to 98.4 mg GAE/g sample. The highest content of total phenolics was observed in *O. sanctum* extract (98.4 mg/g), followed by *M. cordifolia* opiz. extract (62.8 mg/g) and *O. basilicum* (59.0 mg/g), respectively. The ethanolic extracts were also assessed for the antioxidant activity

using the DPPH radical method and expressed as percent DPPH• inhibition (Table I). It was found that *M. cordifolia* opiz. extract showed the strongest antioxidant activity with IC_{50} of 39.0 μg/mL, followed by *O. sanctum* (47.0 μg/mL) and *O. basilicum* (55.6 μg/mL). The strong antioxidant activities of these plant extracts are likely because of their high phenolic contents. This is similar to the results presented in Thitilertdecha's research [11], which suggested that the antioxidant activities of rambutan extracts were remarkably related to their phenolic contents.

TABLE I. TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THE ETHANOLIC EXTRACTS FROM LAMIACEAE PLANTS

sample	Total phenolic content (mg GAE/g sample)	Antioxidant activity (IC_{50} μg/mL)
O. basilicum	59.0 ±4.0[b]	55.6 ±0.1[b]
O. sanctum	98.4 ±0.7[a]	47.0 ±0.7[b]
O. americanum	31.5 ±2.5[c]	100.8 ±0.1[c]
M. cordifolia opiz.	62.8 ±2.6[b]	39.0 ±3.3[b]
Rosmarinic acid	-	8.4 ±1.3[a]
Quercetin	-	21.7 ±3.0[a]

-Values are expressed as means ±SD.
- [a-c] Means in the column followed by different letters are significantly different (P<0.05)

Previous reports have shown that rosmarinic acid, luteolin and luteolin glycosides were generally found in Lamiaceae plants [7], [8]. Therefore, the amounts of rosmarinic acid (RA), luteolin (LU) and apigenin (AP) in the ethanolic extracts were measured using HPLC analysis. The results showed that rosmarinic acid was found in all samples ranging from 1.16 to 8.45 mg/g (Fig. 1). *M. cordifolia* opiz. extract contained high amount of RA (8.45 mg/g), followed by *O. sanctum* extract (4.43 mg/g), *O. americanum* (1.31 mg/g) and *O. basilicum* extract (1.16 mg/g), respectively. Luteolin was found only in *O. sanctum* (0.97 mg/g), while apigenin was not be detected in any of the extracts.

B. *Antiglycation Activities of Ethanolic Extracts from Lamiaceae Plants*

The ability of the ethanolic extracts from Lamiaceae plants to inhibit AGEs formation was evaluated using the BSA-MGO assay and histone-MGO assay. As is well-known, MGO, an intermediate of AGE formation, can induce crosslinking of both extracellular and intracellular proteins in body tissue. BSA which is a serum protein was considered to be an extracellular protein. Histone was chosen an intracellular protein because it contains a very rich of arginine and lysine residues which are targeted for glycation. Fig. 2 (A) shows the inhibitory effect of the ethanolic extracts on AGE generation in histone-MGO model. The *O. sanctum* extract was more effective than the other extracts at a concentration of 250 μg/mL with 43.0% inhibition. It has been observed that the *O. sanctum* extract not only showed the highest in phenolic contents and the antioxidant activity, but also showed the strongest antiglycation property. However, the % AGE inhibition of these extracts were found to be less effective than the antiglycative standards at the concentration of 25 μg/mL (Fig. 2 (B)).

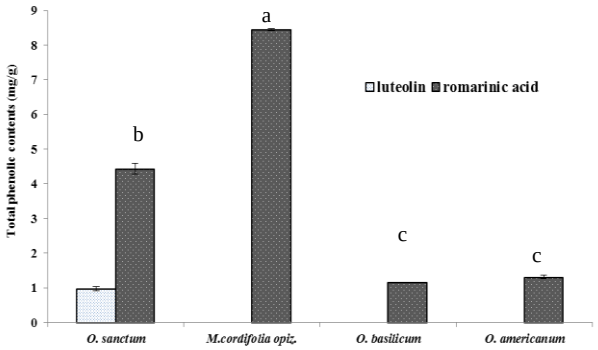
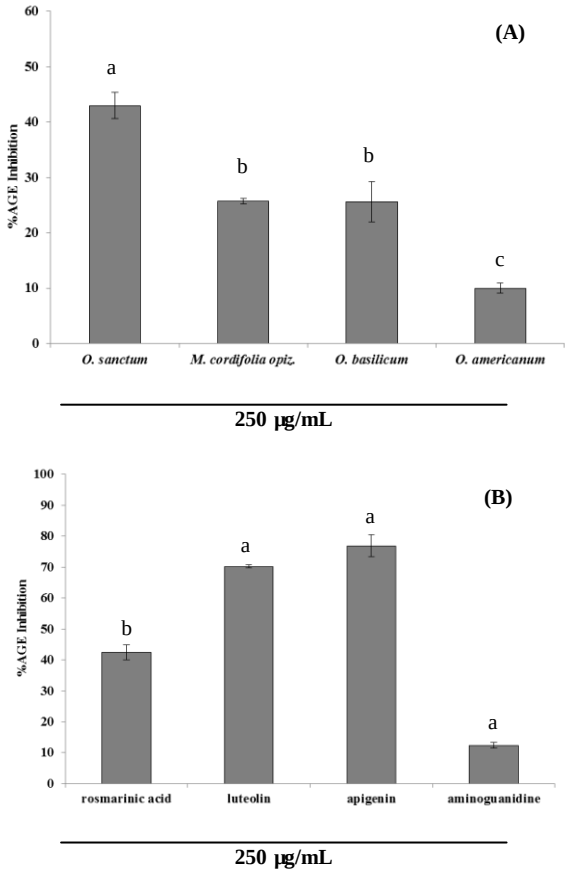


Figure 1. Amount of Rosmarinic acid (RA), Luteolin (LU), Apigenin (AP) in ethanolic extracts from Lamiaceae plants

Fig. 2 (C) displays the effect of four ethanolic extracts on AGE generation after the incubation of BSA with MGO. The *O. sanctum* extract showed the highest inhibition of fluorescence AGE formation with 27.4% inhibition at a concentration of 500 μg/mL. While, both *O. basilicum* and *O. americanum* extracts did not have the anti-glycation activity. The strong antiglycation activities of *O. sanctum* extract are possibly a result of luteolin that are only found in *O. sanctum*. This result was supported by the previous study reporting that luteolin showed more significant inhibitory effect on methylglyoxal-medicated protein modification than other standard flavonoid compounds [13].



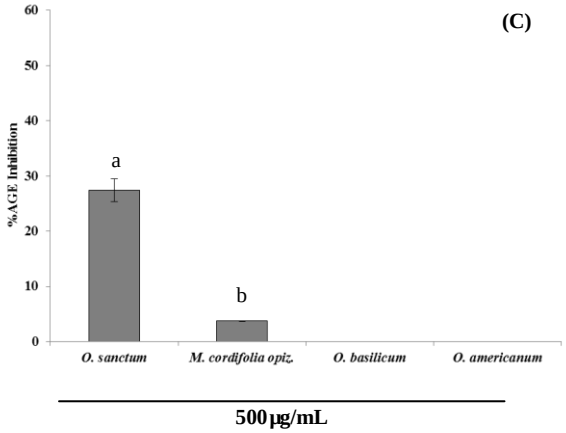


Figure 2. % AGE inhibition of four ethanolic extracts from Lamiaceae plants in different protein models, (A) on histone-MGO model at a concentration of 250 µg/mL, (B) inhibitory effects of 25 µg/mL apigenin, rosmarinic acid, luteolin and aminoguanidine on histone-MGO model, (C) % AGE inhibition of four ethanolic extracts on BSA-MGO model at a concentration of 500 µg/mL

IV. Conclusion

This study shows the investigation of the inhibitory effects of four species of Lamiaceae plants on the formation of advanced glycation endproducts (AGEs) in BSA-MGO and histone-MGO models. The result primarily revealed that the ethanolic extract of *O. sanctum* showed higher content of total phenolic compounds and stronger antioxidant activity when compared with other samples. In addition, *O. sanctum* extract also exhibited potential AGE inhibition in both model proteins. Therefore, *O. sanctum* might potentially be a natural resource of therapeutics against protein glycation and oxidative stress in diabetic patients.

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