APPENDICES

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APPENDIX A

Calculations

A-1 Total phenolic content of culinary plants

The extract solution dissolved with ethanol (1 g/mL) was used. The determination of the total phenolic content was carried out by comparing the absorbance with the standard calibration curve (**Figure A-1**). The calculation can be performed as following.

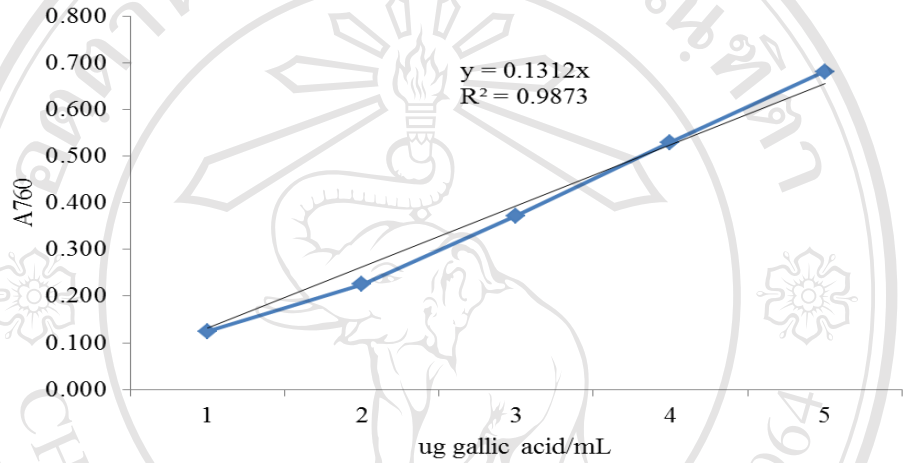

Figure A-1 Standard calibration curve of gallic acid solution.

The ethanolic extract of *Ocimum sanctum* presented the absorbance of 0.660 (y)

The standard calibration's equation was: y = 0.1312x; where y was the absorbance and x was the gallic acid quantity (µg).

Therefore; x = (y)/0.131

Which the absorbance (y) was 0.660

x = (0.660)/0.1312

x = 5.03 µg gallic acid equivalent/mL

the extract diluted 50 times

= 5.03x50

= 251 µg gallic acid equivalent/mL

Therefore 1 mL of the extract (1g/mL) contained phenolic content 251 µg gallic acid

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In summary; The ethanolic extract of *Ocimum sanctum* 1 g contained phenolic compounds 251 µg gallic acid equivalent (251 µg/g extract) or (0.251 mg/g extract).

A-2 Total flavonoid content of culinary plants

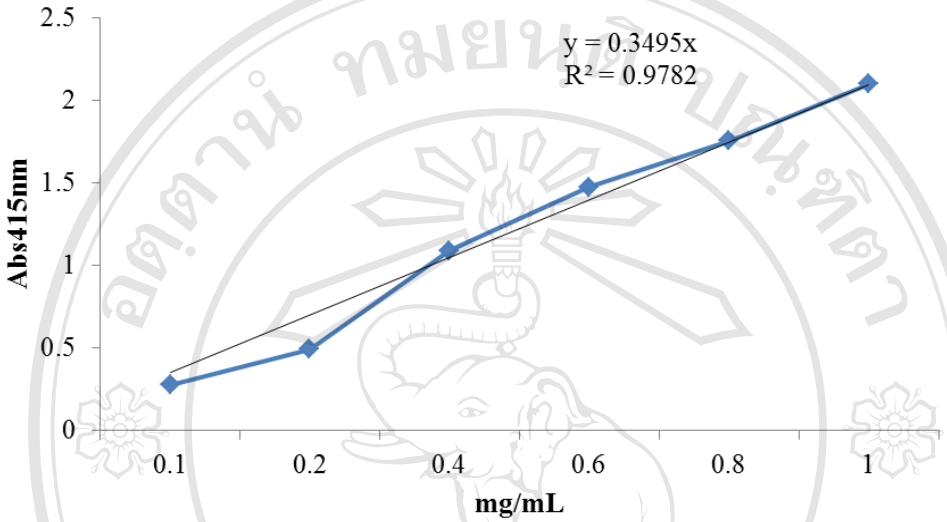

Figure A-2 Standard calibration curve of quercetin solution.

The ethanolic extract of *Ocimum sanctum* presented the absorbance of 0.448 (y)

The standard calibration's equation was: y = 0.3495x; where y was the absorbance and x was the quercetin quantity (mg).

Therefore; x = (y)/0.3495

Which the absorbance (y) was 0.448

x = (0.448)/0.3495

x = 1.28 mg gallic acid equivalent/mL

the extract diluted 50 times

= 1.28x50

= 64.1 mg quercetin equivalent/mL

Therefore 1 mL of the extract (1g/mL) contained phenolic content 64.1 mg quercetin

In summary;

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The ethanolic extract of *Ocimum sanctum* 1 g contained phenolic compounds 64.1mg quercetin equivalent (64.1 mg/g extract) or (0.064 g/g extract).

A-3 Quantification of rosmarinic acid, methyl eugenol, luteolin and apigenin

The extract solution (1 mg/mL) was used. The determination each individual phenolic component, rosmarinic acid, methyl eugenol, luteolin and apigenin was carried out by peak area comparison with calibration curve of rosmarinic acid (**Figure A-3**), luteolin (**Figure A-4**), apigenin (**Figure A-5**) and methyl eugenol(**Figure A-6**) by using HPLC. The calculation can be performed as following.

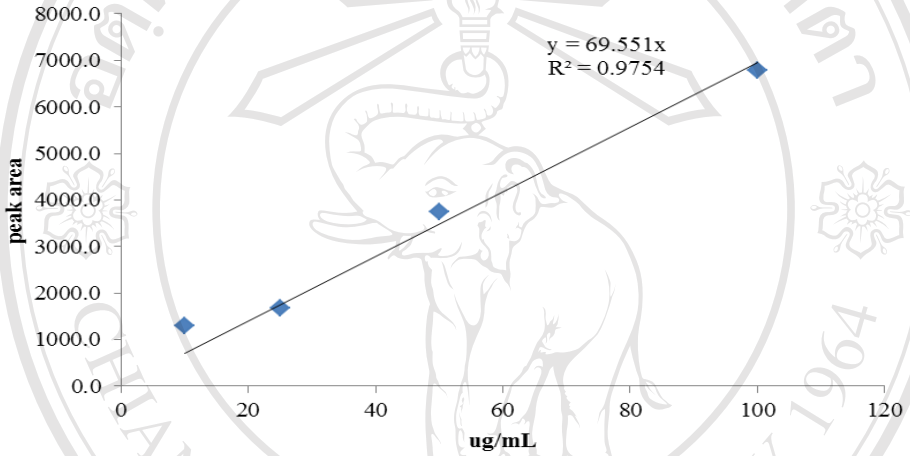

Figure A-3 Calibration of rosmarinic acid

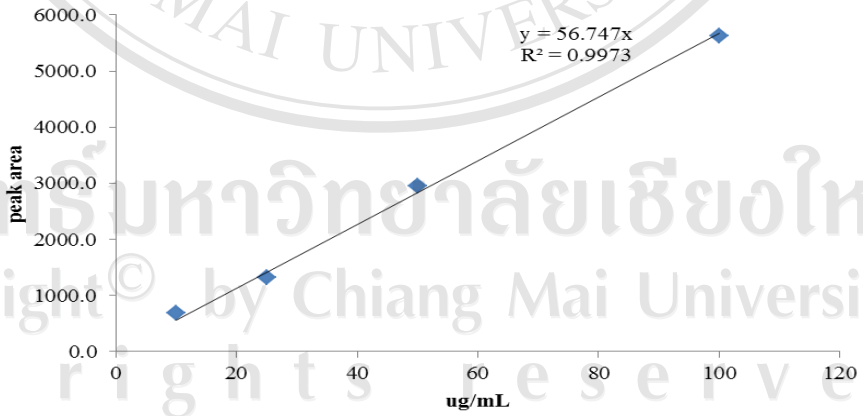

Figure A-4 Calibration of luteolin

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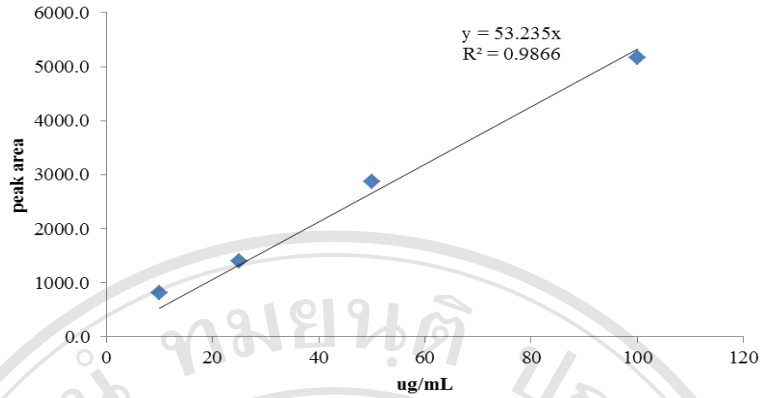

Figure A-5 Calibration of apigenin

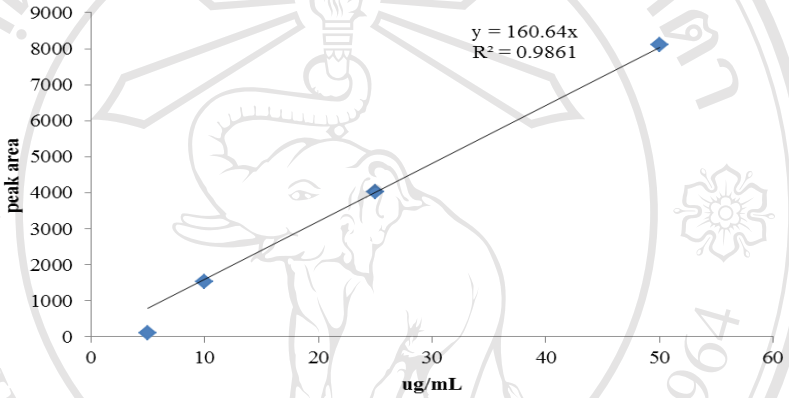

Figure A-6 Calibration of methyl eugenol

For the example of rosmarinic acid quantification, 10 µL (1mg/mL) of the ethyl acetate fraction of *Ocimum sanctum* (purple) presented the peak area of 451.1 (y)

The standard calibration's equation was: y = 69.551x (**Figure A-3**); where y was the peak area and x was the rosmarinic acid concentration (µg/mL).

Therefore; x = (y)/69.551

Which the peak area (y) was 9867.0

x = (451.1)/69.551

x = 6.48 µg rosmarinic acid/mL

10 µL sample contained rosmarinic acid 6.48 µg/mL

From the concentration of sample (1 mg/mL), sample 1 mg contained rosmarinic acid 6.48 µg

In summary The the ethyl acetate fraction of *Ocimum sanctum* (purple) 1 mg contained rosmarinic acid 6.48 µg (6.48 µg /g ethyl acetate (EA) fraction)

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APPENDIX B

Kinetic of α-glucosidase

The double reciprocal plot, 1/V versus 1/[S], is shown below:

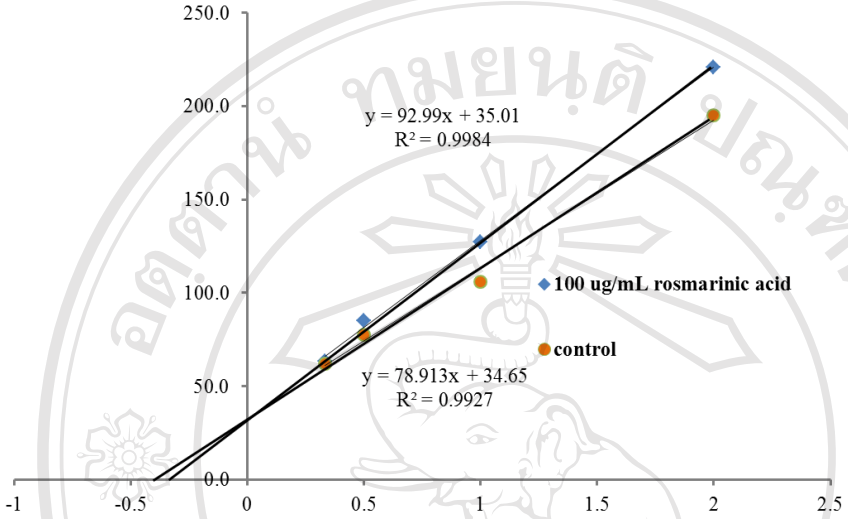

Figure B-1 Linweaver-Burk plot of rosmarinic acid standard

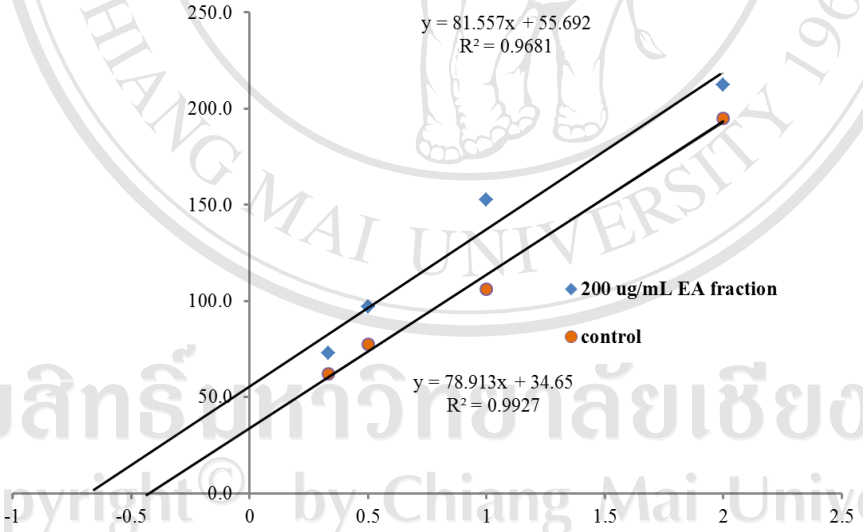

Figure B-1 Linweaver-Burk plot of EA fraction of *Ocimum sanctum* (purple)

Vmax can be obtained from the y-intersect of each line equation, when [S] is in great excess and as 1/[S] approaches zero. At this point, Vmax = 1/y-intersect.

For the example of rosmarinic acid α-glucosidase inhibitory activity,

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Control (without inhibitor)Vmax= 1/y-intersec = 1/34.65 = 0.0286 mM/min

Rosmarinic acid (100µg/mL) Vmax=1/y-intersec = 1/35.01 = 0.0286 mM/min

The affinity of this enzyme for each substrate/inhibitor can be obtained from the slope: Km = Slope/Vmax

Control(without inhibitor) Km = Slope/Vmax = 78.913/0.0286 = 2.76 mM

Rosmarinic acid (100µg/mL) Km = Slope/Vmax = 92.99/0.0286 = 3.25 mM

When the concentration of inhibitor increases, Km value are changed but Vmax are constant; thus the nihibition type of rosmarinic acid is a mixed-typed inhibitor.

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APPENDIX C

Supporting publications

Chiang Mai J. Sci. 2014; 41(1) : 105-116
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Antioxidant and Antiglycation Activities of Some Edible and Medicinal Plants

Khwanta Kaewnarin [a,b], Hataichanoke Niamsup [a,b], Lalida Shank [a,b] and Nuansri Rakariyatham [a,b]*

[a] The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

[b] Division of Biotechnology, Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand.

*Author for correspondence; e-mail: nuansri1@yahoo.com

Received: 14 November 2012
Accepted: 19 March 2013

ABSTRACT

Protein glycation and oxidative stress caused by chronic hyperglycemia are the major factors in diabetic complications. In the attempt to search for natural remedies, ethyl acetate and ethanol extracts from twenty Thai edible and medicinal plants were assessed in terms of their phenolic and flavonoid contents as well as their antioxidant and antiglycation activities. The highest amounts of phenolic and flavonoid compounds were found in the ethanolic extract of the young leaves of *Punica granatum* followed by those of *Dimorcarpus longan* and *Mangifera indica*, respectively. These three plant extracts also exhibited the highest antioxidant activity. A high correlation between the antiglycation activity and the phenolic and flavonoid contents was observed in all extracts. In addition, five ethanolic extracts—from *Tamarindus indica, Psidium guajava, Mangifera indica, Dimocarpus longan* and *Punica granatum* young leaves—were determined for their concentrations required to inhibit 50% (IC_{50}) of either glucose or methyl glyoxal-derived glycation. *P.granatum, M.indica* and *P.guajava* extracts showed high antiglycation activity in the BSA-glucose model, with IC_{50} values of 110.4, 214.4 μg/mL and 243.3 μg/mL, respectively. The IC_{50} values of antiglycation activity in the BSA-methylglyoxal model of *M.indica* (54.1 μg/mL), *P.granatum* (69.1 μg/mL) and *D.longan* (74.2 μg/mL) were higher than that of the standard AGE inhibitor, aminoguanidine (91.2 μg/mL). These results indicated that some Thai edible and medicinal plants possessed high contents of phenolic and flavonoid and have potential applications towards the prevention of glycation-associated diabetic complications.

Keywords: antioxidant, antiglycation, diabetic complications

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1. INTRODUCTION

At the present, the number of diabetic patients has rapidly increased, especially in the Asia-Pacific region [1]. Diabetes mellitus, a disorder characterized by hyperglycemia, is caused by insulin deficiency and/or insulin resistance. Prolonged hyperglycemia plays a vital role in the development of chronic diabetic complications such as retinopathy,

cataracts, atherosclerosis, neuropathy, impaired wounding and aging [2-4]. Numerous studies on diabetes have reported that hyperglycemia involves oxidative stress via glucose autooxidation and an interruption of the electron transport chain. Glucose autooxidation catalyzed by transition metals can generate superoxide radical ($O_2^{\cdot-}$) and ketoaldehyde; by which the superoxide radical will be converted to hydroxyl radical ($OH^{\cdot}$) through the Fenton reaction [5-8]. The accelerated oxidation can result in cell damage and induction of specific signaling pathway, for example, the nuclear factor-κB (NF-κB) leading to pro-inflammatory cytokines [9-10]. The protein glycation is a key molecular basis of diabetic complications which results from chronic hyperglycemia. In terms of the glycation mechanism, the carbonyl group of reducing sugars reacts non-enzymatically with the amino group of proteins, nucleic acids and others molecules [11-12] in order to initiate glycation (Amadori or fructosamine products). Subsequently, Amadori products undergo a series of irreversible reactions forming highly reactive carbonyl species (RCS), such as glyoxal, methylglyoxal and 3-deoxy-glucosone [13]. Finally, these reactive carbonyls react with the amino, sulfhydryl and guanidine functional groups of intracellular and extracellular proteins to form the stable advanced glycation endproducts (AGEs). The reactive carbonyl species can also be produced from sugar glyoxidation contributing to the AGE formation [14-15]. AGE products can cross-link with long-lived proteins such as collagen, lens crystallins, and other biological molecules--haemoglobin, low-density lipoprotein---leading to the altered structures and functions of these proteins *in vivo* [16-17]. Ahmed [18] reported that the glycation of lens crystallins has been considered as one of the major factors in causing diabetic cataracts. Furthermore, one of the most well-known AGEs contributing towards diabetic atherosclerosis is glycated-low density lipoprotein (LDL) [8, 19].

In recent years, many synthetic AGEs inhibitors have been found to be effective against AGEs formation, such as aminoguanidine (AG), the most well-known synthetic prodrug. However, their practical applications are limited because of their toxicity and severe side effects [12, 20]. Besides, some AGEs inhibitors contribute to the pyridoxal sequestration causing vitamin B6 deficiency in diabetic patients [11]. Currently, many plant extracts and purified constituents have been demonstrated as able to suppress AGE formation. Procyanidins, extracted from cinnamon [12], as well as caffeic acid and chlorogenic acid from mate tea extracts [21], were shown to be the active constituents responsible for the antiglycation effect. Additionally, several scientific reports have revealed that the antiglycation of plant extracts can be attributed to the phenolic compounds, which are correlated with their free radical scavenging activities [12, 17, 22-25]. In previous studies, our teams have investigated several Thai edible plants which contain large amounts of bioactive compounds, particularly phenolic compounds that exhibit strong antioxidant activities [26-27]. However, phytochemical data of compounds involved in alleviating or preventing diabetic complications are still needed. For these reasons, this study aims to evaluate the antioxidant and antiglycation activities of various edible and medicinal plants including the correlations with their total phenolic and flavonoid contents.

2. MATERIALS AND METHODS

2.1 Plant Materials and the Preparation of Crude Extracts

Plant materials (Table 1) were purchased

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from the local market in Chiang Mai, Thailand during the period of April to August, 2011. The plant materials were dried at 50°C and powdered. The extraction was prepared as described by Harborne [28] with slight modifications. Three grams of each sample were extracted with ethyl acetate (50 mL, x3) over 1 h in a shaker at room temperature. Ethyl acetate (EA) extract was filtered through Whatman's no. 1 filter paper. The dried residue was then successively extracted with 80% (v/v) ethanol (50 mL, x3). After filtration, the ethyl acetate and ethanolic extracts (ET) were filtered and allowed to evaporate and lyophillize.

Table 1. Edible and medicinal plants used in this study.

	Common name	Extracted part
Allium cepa	Onion	Whole bulb
Allium ascalonicum	Shallot	Whole bulb
Allium sativum	Garlic	Whole bulb
Gynura divaricata	-	Leaves
Gymnema inodorum	-	Leaves
Coccinia grandis	ivy gourd	Leaves
Gynostemma pentaphyllum	jiaogulan	Leaves
Coriandrum sativum	coriander	Leaves
Apium graveolens	chinese celery	Leaves
Eryngium foetidum	false coriander	Leaves
Centella asiatica Urban	Asiatic pennywort	Leaves
Cissus quadrangularis	-	Stems
Andrographis paniculata Wallex Nees	king of bitter	Leaves
Clitoria ternatea	blue pea	Flowers
Musa sapientum	banana	Flowers
Tamarindus indica	tamarind	Young leaves
Psidium guajava	guava	Young leaves
Mangifera indica	mango	Young leaves
Dimocarpus longan	longan	Young leaves
Punica granatum	pomegranate	Young leaves

2.2 Determination of Total Phenolic Content

The total phenolic content of each extract was assessed by the Folin-Ciocalteu method with some modifications [26] and gallic acid was used as the standard phenolic compound. The extract which was redissolved in ethanol (100 µL) was transferred to a test tube containing 7.9 mL of distilled water. The samples were mixed with 500 µL of the Folin-Ciocalteu reagent and left to react for 5 min. The reaction mixture was neutralized with the addition of 1.5 mL of 200g/L sodium carbonate (Na_2CO_3), followed by 2 h incubation with constant shaking. The absorbance was then measured at 760 nm. The total phenolic content was expressed as mg gallic acid equivalent (GAE)/g sample.

2.3 Determination of Total Flavonoid Content

Total flavonoid content was determined by a colorimetric method [29] with slight modifications and quercetin was used as

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the standard flavonoid. One half mL of the extract was mixed with 2 mL of distilled water, followed by addition of 0.15 mL of 50 g/L sodium nitrite ($NaNO_2$). After 5 min of reaction, 0.15 ml of 100 g/L aluminium chloride ($AlCl_3$) solution was added. The reaction solution was mixed well and incubated at room temperature for 15 min, and the absorbance at 415 nm was measured. Total flavonoid content was expressed as µg quercetin equivalent (QE)/g sample.

2.4 *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 [27]. One mL of DPPH radical solution (0.1 mM $DPPH^{\cdot}$ in methanol) was well mixed with 3 mL 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 517nm. The percent DPPH radical scavenging activity was calculated using the following formula:

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

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

2.5 *In vitro* Determination of Antiglycation Activity in BSA-glucose Model

Inhibition of Protein glycation method was performed according to Matsuura et al. [30] with some modifications. The reaction mixture (2 mL) contained 800 µg/mL bovine serum albumin (BSA), 200 mM D-glucose and with/without the extract (1 mg/mL) in phosphate buffer (50 mM, pH 7.4) in the presence of 0.2g/L of sodium azide (NaN_3). The reaction mixture was incubated at 37°C for 7 days. 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 spectrofluorometer. Aminoguanidine (AG) (1 mg/mL) was used as a positive control. Results were expressed as percent AGE inhibition 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 mixtures, respectively. Different extract concentrations (50-500 µg/mL) providing 50% AGE inhibition (IC_{50}) were calculated from the graph of inhibition percentage against the extract concentration.

2.6 *In vitro* Determination of Antiglycation Activity in the BSA-methylglyoxal Model

The evaluation for the inhibition of the middle stage of protein was performed according to Peng et al. [12]. 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-methylglyoxal reaction mixture was incubated at 37°C for 3 days with/without various concentrations (50-500 µg/mL) of the selected plant extracts. Aminoguanidine (AG) (10-100 µg/mL) was used as a positive control. The fluorescence intensity was measured at an excitation wavelength of 370 nm and an emission wavelength of 420 nm with a Perkin Elmer LS-50B. The percentage of the AGE inhibition was calculated using the same equation as in the BSA-glucose model.

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2.7 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. The correlation (r) between the two variants was analyzed using the Pearson test. All statistical analyses were performed using SPSS software (SPSS 17.0 for windows; SPSS Inc., Chicago).

3. RESULTS AND DISCUSSION

3.1 Total Phenolic and Total Flavonoid Contents

This study involved twenty Thai edible and medicinal plants that are regularly consumed and applied in traditional forms of medicines in Thailand. Total phenolic and flavonoid contents, antioxidant and antiglycation activities of the extracts of these plants were determined. The phenolic content was determined by the Folin-Ciocalteu method and expressed as mg gallic acid equivalent (GAE) per g of dry sample. Table 2 shows the content of phenolic compounds in various plant extracts ranging from 0.02 to 3.13 mg/g sample. Significant differences ($p < 0.05$) were found in all of these amounts. High amounts of phenolic compounds were found in ethanolic (ET) fractions of *P.granatum* (3.13 mg/g), *D.longan* (1.68 mg/g), *M.indica* (1.51 mg/g) and the ethyl acetate (EA) fractions of *D.longan* (1.20 mg/g) and *P.granatum* (1.11 mg/g), respectively. The total flavonoid content in each plant extract was also determined using a colorimetric method and reported as the μg quercetin equivalent (QE) per g of dried sample. The results showed that the content of the flavonoid range from 1.39 to 237 mg/g. Significantly, the highest amount of flavonoids was shown ($p < 0.05$) in the ET fraction of *P.granatum* (237 mg/g) followed by *D.longan* (160 mg/g), *M.indica* (151 mg/g) and EA fractions of *D.longan* (136 mg/g) and *M.indica* (135 mg/g). It could be observed that the young leaf extract of *P.granatum* exhibited the highest amounts of total phenolics and flavonoids. Moreover, the ethanolic extracts of *P.granatum, D.longan* and *M.indica* contained higher amounts of phenolic compounds and flavonoids than their ethyl acetate (EA) extracts. The results correspond with Harborne's work [28] which reported that alcohol is a suitable organic solvent for phenolic and flavonoid extraction.

Table 2. Total phenolic and total flavonoid contents in the ethyl acetate (EA) and ethanolic (ET) extracts of edible and medicinal plants.

Plants	Total phenolic content (mg GAE/g)		Total flavonoid content (μg QE/g)	
	EA extract	ET extract	EA extract	ET extract
A.cepa	0.04±0.0	0.04±0.0	2.96±0.2	8.49±0.2
A.ascalonicum	0.04±0.0	0.10±0.0	3.82±1.0	6.16±0.2
A.sativum	ND	0.02±0.0	2.96±0.4	1.39±0.0
G.divaricata	0.16±0.0	0.40±0.0	48.3±6.5	78.6±7.2[d]
G.inodorum	0.08±0.0	0.38±0.0	50.4±1.4	159±6.4[b,c]
C.grandis	0.07±0.0	0.18±0.0	68.4±4.3	51.3±4.9
G.pentaphyllum	0.16±0.0	0.13±0.0	49.0±5.1	26.3±3.2
C.sativum	0.05±0.0	0.09±0.0	22.2±0.8	15.4±0.4
A.graveolens	0.03±0.0	0.14±0.0	27.8±1.4	40.5±0.7
E.foetidum	0.05±0.0	0.07±0.0	19.0±1.0	9.26±2.0

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Table 2. (Continue)

Plants	Total phenolic content (mg GAE/g)		Total flavonoid content (μg QE/g)	
	EA extract	ET extract	EA extract	ET extract
C.asiatica Urban	0.04±0.0	0.16±0.0	19.9±1.7	34.5±5.1
C.quadrangularis	0.03±0.0	0.04±0.0	18.2±1.4	5.54±1.0
A.paniculata Wallex Nees	0.03±0.0	0.05±0.0	118±0.6^{c}	13.5±0.5
C.ternatea	0.12±0.0	0.26±0.0	50.3±1.6	76.6±2.8
M.sapientum	0.03±0.0	0.11±0.0	9.46±0.9	8.67±1.3
T.indica	0.29±0.0^{d}	0.15±0.0	130±3.9^{b}	69.3±1.7
P.guajava	0.14±0.0	0.69±0.1^{d}	93.6±2.6^{d}	73.4±5.3
M.indica	0.74±0.0^{c}	1.51±0.0^{c}	135±4.6^{a,b}	151±4.7^{c}
D.longan	1.20±0.1^{a}	1.68±0.2^{b}	136±5.5^{a}	160±2.1^{b}
P.granatum	1.11±0.1^{b}	3.13±0.1^{a}	33.2±1.9	237±5.5^{a}

- Values are expressed as means ± SD.
- $^{a\text{-}d}$ Means in the column followed by different letters are significantly different *($p < 0.05$)*
- ND = not determined

3.2 Antioxidant Activity

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable free radical which is frequently used in measuring antioxidant activities due to the following strengths: its direct measurement of inhibition, simplicity and quick analysis [31]. Both solvent extracts were assessed for the antioxidant activity using the DPPH radical method and expressed as percent DPPH· inhibition (Table 3). Significant differences ($p < 0.05$) were found in the antioxidant activity of the plant extracts. Strong antioxidant activity was found in both EA and ET fractions, especially those of *P.granatum* (94.1% and 95.7%), *D.longan* (94.3% and 95.5%), *M.indica* (93.9% and 94.8%) and *P.guajava* (94.6% and 93.5%). The strong antioxidant activities of these plant extracts are possible a result of the high contents of phenolics and flavonoids which have been shown to be highly antioxidant [32-35]. Correlations between the antioxidant activity and the phenolic and flavonoid contents were investigated (Table 4). There were strong correlations ($r_{ET} = 0.779$ and $r_{EA} = 0.866, p < 0.05$) between antioxidant activity and phenolic content for all ethanolic (ET) and ethyl acetate (EA) extracts. This relationship indicated that the free radical scavenging activity of the plant extracts was associated with the phenolic compounds. This result agreed with previous studies reporting that phenolic compounds in various plant extracts are the major constituents with free radical scavenging property to donate a hydrogen atom from their phenolic hydroxyl groups [25, 36-39]. This is similar to the results presented in Thitilertdecha's research [27], which suggested that the antioxidant activities of rambutan extracts were remarkably related to their phenolic contents. Additionally, high correlation ($r_{ET} = 0.796$) was observed between antioxidant activity and the flavonoid content in the ethanolic fractions of all plants. Moderate correlation ($r_{EA} = 0.583$) was observed for their ethyl acetate (EA) fractions. The findings showed that ethanol was a good solvent for the extraction of antioxidant substances. These correlations suggest that the strong antioxidant activity present in these plants possibly come from the phenolic compounds.

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Table 3. Antioxidant and antiglycation activities of EA and ET plant extracts.

Plants	DPPH radical scavenging activity (% Inhibition)		Antiglycation activity (% Inhibition)	
	EA extract	ET extract	EA extract	ET extract
A.cepa	1.73±0.4	3.34±0.8	58.1±6.6	71.7±0.4
A.ascalonicum	2.91±0.3	3.04±0.8	49.8±1.7	78.7±1.3
A.sativum	ND	ND	7.19±1.4	8.06±1.8
G.divaricata	31.3±0.1	60.7±3.4^{b}	97.6±0.7^{b,c}	91.6±0.7^{d}
G.inodorum	87.9±0.7^{b}	53.3±1.3	97.6±0.1^{b,c}	99.5±0.2^{a}
C.grandis	ND	35.2±2.7	99.6±0.3^{a}	95.9±2.1^{c}
G.pentaphyllum	11.0±0.6	ND	98.5±0.5^{a,b}	96.3±0.2^{a}
C.sativum	6.64±0.1	28.6±0.4	82.3±2.9	85.7±2.4^{d}
A.graveolens	1.91±0.1	18.7±0.6	88.0±0.6	99.6±0.2^{a}
E.foetidum	8.82±0.4	8.87±0.5	81.0±1.1	61.8±1.6
C.asiatica Urban	9.64±0.3	59.7±4.8^{b}	82.2±4.7	95.6±1.8^{c}
C.quadrangularis	4.00±0.2	6.45±1.7	80.6±0.6	56.3±3.1
A.paniculata Wallex Nees	0.64±0.1	11.5±0.4	89.6±0.6^{d}	96.5±0.9^{b,c}
C.ternatea	12.6±1.0	28.3±0.5	98.4±0.2^{a,b}	99.9±0.4^{a}
M.sapientum	6.12±0.1	15.8±3.1	45.7±1.3	70.7±0.7
T.indica	23.4±1.8	17.6±1.1	99.4±0.4^{a}	96.2±0.1^{c}
P.guajava	94.6±0.2^{a}	93.5±0.9^{a}	99.8±0.1^{a}	99.8±0.0^{a}
M.indica	93.9±0.5^{a}	94.8±0.2^{a}	99.8±0.1^{a}	99.9±0.0^{a}
D.longan	94.3±0.1^{a}	95.5±0.2^{a}	99.9±0.0^{a}	99.8±0.0^{a}
P.granatum	94.1±0.3^{a}	95.7±0.2^{a}	99.8±0.0^{a}	99.0±0.0^{a}

- Values are expressed as means ± SD.
- $^{a-d}$ Means in the column followed by different letters are significantly different *($p < 0.05$)*
- ND = not determined

Table 4. The Pearson correlation coefficient of total phenolic and flavonoid contents with antioxidant and antiglycation activities of plant extracts.

	Correlation			
	Antioxidant activity		Antiglycation activity	
	EA extract	ET extract	EA extract	ET extract
Phenolic content	0.866	0.779	0.849	0.864
Flavonoid content	0.583	0.796	0.879	0.796

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3.3 Antiglycation Activity

The antiglycation activity of plant extracts was evaluated for the inhibition of advanced glycation endproducts(AGEs) formation based on the BSA/glucose system. The results indicated that sixteen plants exhibited potential antiglycation activity (> 80% inhibition) (Table 3). Similarly to the antioxidant activity, strong antiglycation activity was found statistically in both the EA and ET extracts ($p < 0.05$), especially those of *T.indica* (99.4% and 96.2%), *P.guajava* (99.8% and 99.8%), *M.indica* (99.8% and 99.9%), *D.longan* (99.9% and 99.8%) and *P.granatum* (99.8% and 99.0%). This correlation was also evaluated (Table 4). Data revealed substantial correlation of the antiglycation activity of the plant extracts with the phenolic($r_{ET} = 0.864$ and $r_{EA} = 0.849$) and flavonoid contents ($r_{ET} = 0.796$ and $r_{EA} = 0.879$, $p < 0.05$). These results are noteworthy not only because the phenolic and flavonoid contents of these extracts show a positive relationship with the antioxidant activity, but also with the antiglycation property. Many published studies have suggested that the phenolic and flavonoid compounds in plant extracts are responsible for the antiglycation activity [12, 17, 22-25]. For example, it has been reported that cinnamon bark extract could inhibit the formation of AGEs which is mainly attributed to its phenolic constituents, such as catechin, epicatechin, and procyanidin B2.

As a result of their strong antioxidant and antiglycation activities, the ethanolic young leaf extracts of 5 plants (*T.indica, P.guajava, M.indica, D.longan* and *P.granatum*) were selected for further investigation of their antiglycation activity against glucose and methylglyoxal models. In the BSA-glucose model, it was found that *P.granatum* ($IC_{50} = 110$ μg/mL) had significantly stronger inhibitory activity than *M.indica* and *P.guajava* extract ($IC_{50} = 214$ μg/mL and 243 μg/mL), respectively ($p < 0.05$). However, these extracts were found to be less effective than aminoguanidine ($IC_{50} = 50.2$ μg/mL) which is the positive control.

The inhibitory effect of the selected plant extracts on a BSA-methylglyoxal (MGO) model was also reported (Figure 1(B)). BSA-methylglyoxal model represented the middle stage of protein glycation in which sugar is oxidized to α-dicarbonyl compounds such as methylglyoxal, glyoxal and 3-deoxyglucosome, which are more reactive in reacting with amino group of protein leading to AGE formation [17]. The IC_{50} values showed that *M.indica* extract (54.1 μg/mL) had statistically higher antiglycation activity than *P.granatum* and *D.longan* extract (69.1 μg/mL and 74.2 μg/mL, respectively) ($p < 0.05$). In addition, these results indicated that the ethanolic extracts of *M.indica*, *P.granatum* and *D.longan* had significantly higher inhibitory activity-against AGE formation induced by methylglyoxal than aminoguanidine ($IC_{50} = 91.2$ μg/mL) ($p < 0.05$). This is likely the result of the high contents of phenolic and flavonoid compounds in these plant extracts. It has been that reported *P.granatum* leaves contain high amounts of tannins and phenolic compounds [40], whereas Gil [41] has reported the presence of phenolic apigenin and luteolin glycosides in pomegranate leaves [41]. This fact suggests that *P.granatum* leave extracts were responsible for the inhibition of AGE formation in the BSA-methylglyoxal model.

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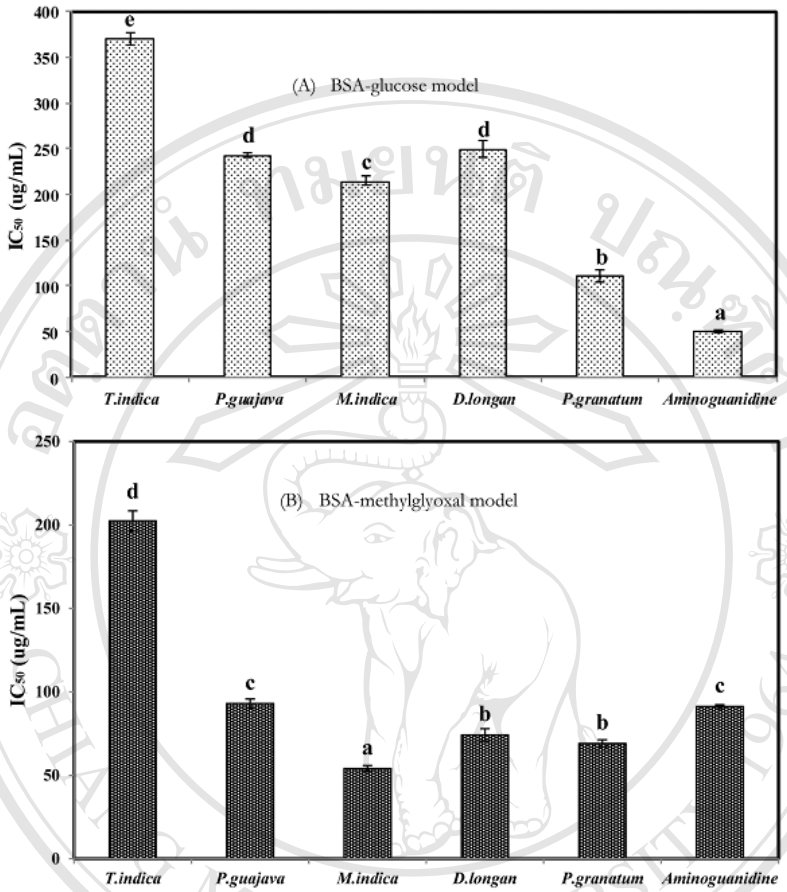

Figure 1. Inhibitory effect of the selected plant extracts (A) on the formation of glycation in BSA- glucose model (B) on the formation of glycation in BSA- methylglyoxal model. Aminoguanidine was used as a positive control. Different superscripts indicate statistically significant differences ($p < 0.05$).

These results were consistent with a previous study [12] on the correlation of the antiglycation activities and the total phenolic contents of bean extracts. Interestingly, the young leaf extracts of *M.indica*, *P.granatum* and *D.longa* displayed significantly greater inhibitory activities than amionguanidine aminoguanidine in the BSA-methylglyoxal model which is likely one of their principle mechanisms of the inhibition in the AGE formation [39]. The previous study demonstrated that several phenolic compounds, such as catechin, epicatechin, and procyanidin B2, and phenol polymers, identified from the subfractions of the aqueous cinnamon extract displayed significant inhibitory effects on the formation of AGEs [12]. Their antiglycation activities were related to their trapping abilities of the reactive carbonyl species, such as methylglyoxal (MGO), an intermediate reactive carbonyl of AGE formation, of which proanthocyanidins (condensed tannins) were shown to be more effective scavenging reactive carbonyl species than other isolated compounds.

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Besides, Wu [24] has reported that flavonoids, especially, luteolin and rutin, developed a more significant inhibitory effect on methylglyoxal-medicated protein modification. While, rutin, quercetin and kaempferol were reported to be effective at the last stage of protein glycation in the BSA-glucose model.

4. CONCLUSIONS

The present study shows an evaluation of the antiglycation and antioxidant properties present in the extracts of 20 edible and medicinal plants. Most of the ethanolic extracts from the plants contained higher phenolics and flavonoids than their ethyl acetate extracts. In addition, the correlation was found between the phytochemical compositions of the extracts and their antiglycation and antioxidant activities. Among these extracts, the ethanolic extracts of *T.indica, P.guajava, M.indica, D.longan* and *P.granatum* young leaves exhibited both strong antiglycation and strong antioxidant activities *in vitro*. The ethanolic extracts of *P.granatum, D.longan* and *M.indica* showed higher antiglycation activity in the BSA-methylglyoxal model than the positive control, aminoguanidine. Therefore, it is possible that these edible and medicinal plants might provide effective natural sources of treatment against the glycation reaction and oxidative stress found in diabetic patients.

ACKNOWLEDGEMENT

The authors are grateful to the Center of Excellence for Innovation in Chemistry (PERCH-CIC), the Commission on Higher Education, Ministry of Education, Department of Chemistry, Faculty of Science and the Graduate School, Chiang Mai University, Chiang Mai, Thailand for the financial support of this research.

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

Khwanta Kaewnarin[1], Lalida Shank[2], Hataichanoke Niamsup[3], and Nuansri Rakariyatham[4]
Department of Chemistry and Center of Excellence for Innovation in Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand
Email: [1]khwanta55@gmail.com; [2]lalida_shank@yahoo.com; [3]hataichanoke.n@cmu.ac.th; [4]nuansri1@yahoo.com

***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 250μg/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

Manuscript received May 18, 2013; revised July 15, 2013.

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doi: 10.12720/jomb.2.4.224-227

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, 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 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°C for 4 days with and without the plant extracts of 500µg/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°C for 4 days with the plant extracts of 250µg/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

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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 39.0µg/mL, followed by *O. sanctum* (47.0µg/mL) and *O. basilicum* (55.6µg/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 250µg/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)).

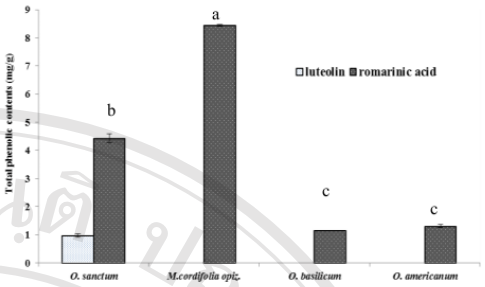
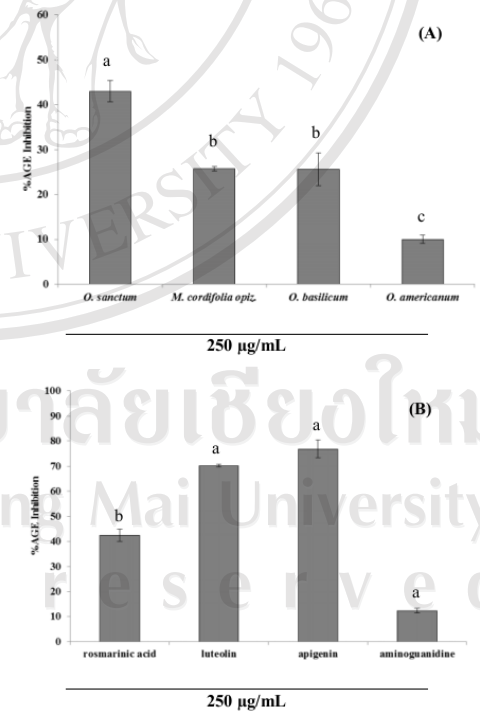


Figure 1. Amount of Rosmarinic acid (RA), Luteolin (LU), Apigenin (AP) in ethanolic extracts from Lamiaceae plants

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 500µg/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].



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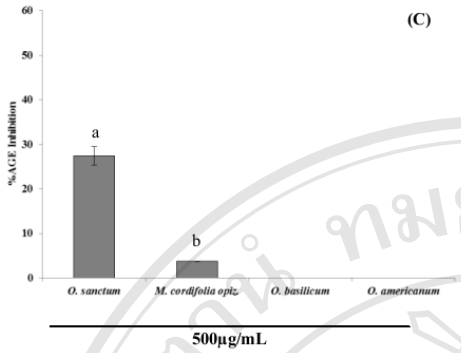

Figure 2. % AGE inhibition of four ethanolic extracts from Lamiaceae plants in different protein models, (A) on histone-MGO model at a concentration of 250 µg/mL, (B) inhibitory effects of 25µg/mL apigenin, rosmarinic acid, luteolin and aminoguanidine on histone-MGO model, (C) % AGE inhibition of four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

Acknowledgements

We are grateful to Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and Department of Chemistry, Faculty of Science and Graduate School, Chiang Mai University, Thailand for the financial support.

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Khwanta Kaewnarin is a Ph.D. student in Biotechnology at Chiang Mai University. She received the B.Sc. degree in Biochemistry and Biochemical Technology (2005) and M.Sc. degree in Biotechnology (2008) from Chiang Mai University, Chiang Mai, Thailand. Her currently research interests include bioactive compounds and natural products.

Hataichanoke Niamsup received her 1st class honor Bachelor degree in chemistry (1991) from Chiang Mai University and her Ph.D. in biochemistry (1995) from University of Illinois at Urbana-Champaign, USA. She is currently appointed as Assistant Professor at Chiang Mai University. Her research interest is application of molecular biology and bioch- emistry in agriculture.

Lalida P. Shank received her M.Sc. and Ph.D. from Department of Biochemistry, Kansas State University, Manhattan, Kansas, USA in 1998 and 2002, respectively. She joined Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, Thailand in 2003. She is currently an assistant professor and enjoys working on collaborative research projects involving enzymology, protein chemistry and applications of proteins and secondary metabolites.

Nuansri Rakariyatham received Doctoral degree in "Doctrate De Troisieme Cycle EN Nutrition et Alimentation" from Bordeaux University, France in 1984. She has specialized in researching the application of bioactive compounds.

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Proceedings
International Conference on Biotechnology for Healthy Living

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The 22nd Annual Meeting of the Thai Society for Biotechnology
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Prince of Songkla University, Trang Campus, Thailand, October 20-22, 2010

Screening for antioxidant and antiglycation properties of Thai indigenous plants

Khwanta Kaewnarin[a] and Nuansri Rakariyatham[b]*
[a] *Division of Biotechnology, Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand*
[b] *PERCH-CIC, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*

Abstract

Chronic hyperglycemia causes induced protein glycation and oxidative stress which is the main determinant of diabetic complications. In this study, Thai indigenous plants which had been reported as having a high natural antioxidant activity were screened for their capability to inhibit oxidative stress and the glycation process. The methanolic extracts of various plants were assessed for their antioxidant activities by using lipid peroxidation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging methods. Their antiglycation activities were also determined through the inhibition of bovine serum albumin (BSA)-glucose model system. The results suggested that six methanolic extracts of *Leucaena leucocephala* , *Morus alba*, *Tamarind indica*, *Gymnema inodorum* Decne. and *Clitoria ternatea* showed high DPPH free radical scavenging activity with the IC_{50} of 5.21, 13.0, 38.8, 46.2 and 56.6 µg/ml, respectively. Furthermore, the lipid peroxidation inhibition of *Morus alba* leave extract had the highest activity (99.1%) followed by *Clitoria ternatea* (97.4%), *Allium ascalonicum* (96.8%) and *Carthamus tinctorius* Linn. (92.2%), respectively. The antiglycation activity of three methanolic extracts of *Tamarind indica Allium ascalonicum, Allium sativum* and *Clitoria ternatea* revealed a strong activity against the formation of advanced glycation endproduct induced by glucose. These results suggest that the methanolic extracts of Thai indigenous plants in this study have possibility to be applied toward the prevention of glycation-associated complications in patients with diabetes.

Keywords: Thai indigenous plants, antioxidant activity, antiglycation, diabetic complications

*Corresponding author, Tel.: +66 53 943341; Fax.: +66 53 892277
E-mail: nuansri1@yahoo.com

Introduction

Chronic hyperglycemia occurring in those who have diabetes has been shown to cause an increase in protein glycation and oxidative damage in biological systems. Protein glycation is a well-known reaction occurring between reducing sugars and amino groups in proteins, lipids or nucleic acids leading to the accumulation of advanced glycation end-products (AGEs). The AGE accumulation has been implicated as a major pathogenic process in diabetic complications and other health disorders such as atherosclerosis and aging. The investigation of AGE inhibitors offers an effective therapeutic approach for the prevention of diabetic complications. Aminoguanidine (AG), a synthetic AGE inhibitor in clinical trials, has displayed strong AGE inhibitory activities, but a concern is that it can lead to severe side effects.

Many Thai plants are known to possess immense medicinal properties such as antimicrobial, anticarcinogenicity and anti-inflammatory activities, as well as antioxidant

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and antiglycation properties. These plants could be used to produce alternative substances used to reduce the risks of diabetes. Therefore, the active compounds from those plants and their related pathways on oxidative stress and protein glycation activities are of great interest. In this study, Thai indigenous plants have been screened for their antioxidant and antiglycation properties, which can be considered of great importance in the very near future.

Materials and Methods

Preparation of Extract

Plant materials were dried in a hot-air dryer at 50 °C until the stability of their dry weight was observed, and they were then ground into powder using a blender. Each powdered plant material was soaked in 95% (v/v) methanol at a ratio of 1:2 for 24 hours at room temperature. The extracted solvent was separated and filtered through Whatman No. 4 filter paper. The residue was then re-extracted again with methanol. After filtration, the combined methanolic extracts were evaporated under a vacuum to absolute dryness and lyophilized. The powdery crude extracts were weighed and stored at 4 °C until they were needed.

Determination of plant extract yield

The yield of freeze-dried extracts based on wet weight basis was calculated from Eq. (1) and is shown below:

$$\text{Yield (\%)} = (W_1 \times 100)/ W_2 \qquad (1)$$

Where W_1 was the weight of extract after freeze-drying and W_2 was the weight of the fresh plant sample.

Determination of anitioxidant activity

Radical DPPH scavenging activity

The free radical scavenging activity of plant extracts was measured by the 1,1-diphenyl-2-picryl-hydrazil (DPPH) method proposed by Blois (1958). 0.1 ml of DPPH in methanol was added to 3 ml of methanolic plant extracts at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm.

$$\text{DPPH Scavenging Effect (\%)} = [(A_o - A_1)/A_o]x100$$

Where A_o represents the absorbance of the control reaction and A_1 represents the absorbance in the presence of the sample extracts.

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Antioxidant activity assay using linoleic acid peroxidation

The antioxidant activity of plant extracts was assayed using the thiocyanate method (Jayprakasha et al., 2001). The crude extracts were dissolved in methanol and stored at 4 °C until they were used. The linoleic acid emulsion was prepared by mixing 0.284 g of linoleic acid, 0.284 g of Tween40 as emulsifier, and 50 ml phosphate buffer. The samples (0.5 ml) were mixed with 2.5 ml of linoleic acid emulsion and 2.5 ml of phosphate buffer (0.2 M, pH 7.0) and incubated at 37 °C for 109 h. Aliquots of 0.1 ml were taken from the incubation mixture at an interval of 6 h and mixed with 2.0 ml of 75% ethanol, 30 µl of 30% ammonium thiocyanate solution and 30 µl of 0.02 M ferrous chloride in 3.5% HCl. After 3 min, the peroxide values were determined by measuring the absorbance at 500 nm using a spectrophotometer. The percent inhibition of lipid peroxidation was calculated using the following fomula:

$$(\%)\ \text{Inhibition} = [(A_o - A_1)/A_o]x100$$

Where A_o represents the absorbance of the control reaction and A_1 represents the absorbance in the presence of the sample extracts.

Measurement of the glycation inhibitory activity

The procedure of Matsuura et *al*. (2002) was modified to measure the glycation inhibitory activity. 2 ml of reaction mixture was prepared containing 800 µg/ml bovine serum albumin, 200 mM D-glucose and with or without 40 µl of plant extracts in 50 mM phosphate buffer, pH 7.4, and then the reaction mixture was incubated at 37 °C in a water bath for 7 days. The fluorescence intensity was measured at an excitation of 370 nm and an emission of 440 nm with a spectofluorometer.

Results and Discussion

The antioxidant and antiglycation properties of 16 Thai indigenous plants were assessed. The extraction yield (g/100g of dried sample) of various plants is presented in Table 1. The extracts were determined for their antioxidant activities. The free radical scavenging capacity of the extracts against common free radicals (DPPH) *in vitro* was investigated. Figure 1 shows the DPPH free radical scavenging activity of these plants. The results indicated that the methanolic extract of *Leucaena leucocephala* had the highest activity with IC_{50} 5.21 µg/ml, followed by the methanolic extract of *Morus alba* (13.0 µg/ml), *Tamarind indica* (38.8 µg/ml), *Gymnema inodorum* Decne. (46.2 µg/ml) and *Clitoria ternatea* (56.6 µg/ml), while all other extracts had lower activity than these plants. When compared to standard quercetin and ascorbic acid, the methanolic extract of *Leucaena leucocephala* showed a higher activity than that of antioxidant standards

Table 1. Extraction yield of methanolic extracts obtained from various plant parts of Thai indigenous plants

Scientific name	Plant part	Yield(%)
1. *Allium cepa*	Whole fruit	3.5
2. *Allium ascalonicum*	Whole fruit	5.4
3. *Allium sativum*	Whole fruit	2.6
4. *Citrus hystrix*	Fruit peel	3.7
5. *Citrus auramtifolia*	Fruit peel	4.0
6. *Citrus sinensis* Osb.	Fruit peel	6.6
7. *Tamarind indica*	Young leave	3.9
8. *Clitoria ternatea*	Flower	6.6
9. *Leucaena leucocephala*	Fresh leave	3.2
10. *Morus alba*	Fruit	6.5
	Dried leave	2.0
11. *Carthamus tinctorius Linn.*	Dried flower	18.3
12. *Gynura divaricata*	Fresh leave	1.3
13. *Stevia rebaudiana*	Fresh leave	14.7
14. *Gymnema inodorum Decne.*	Fresh leave	3.9

The total antioxidant activity by lipid peroxidation based on the ferric thiocyanate method was also evaluated. The methanolic extract of *Morus alba* leaves showed the highest percent inhibition (99.1%), followed by *Clitoria ternatea* (97.4%), *Allium*

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ascalonicum (96.8%) and *Carthamus tinctorius* Linn. (92.2%), respectively. This result exhibited potent antioxidant activity of the methanolic extract from *Morus alba* leaves when compared with the antioxidant standard quercetin (see Figure 2).

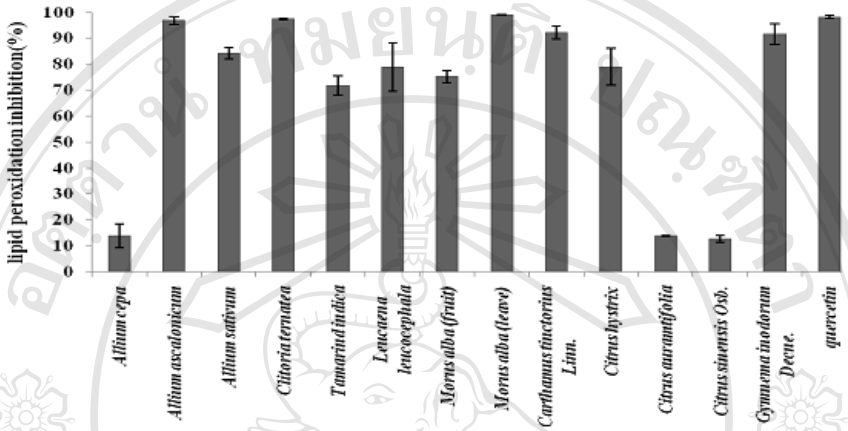

Figure 2. Antioxidant activity of methanolic plant extracts in the lipid peroxidation method.

The antiglycation activity of the plant extracts against advanced glycation endproduct (AGEs) formation induced by glucose-BSA model was evaluated. The methanolic extracts of *Tamarind indica*, *Allium ascalonicum, Allium sativum* and *Clitoria ternatea* were able to achieve more than 50% inhibition against the formation of AGEs induced by glucose at the concentration of 1 mg/ml with the percent inhibition of 67.4%, 53.4%, 52.97% and 50.16%, respectively (see Figure 3).

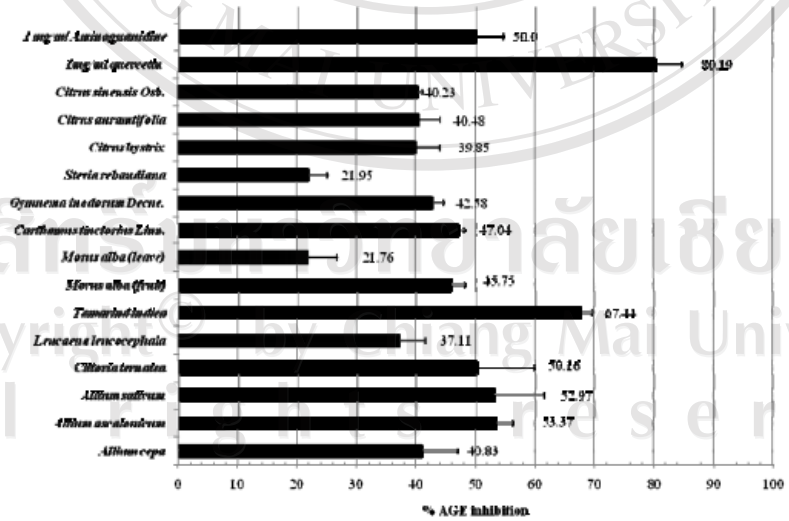

Figure 3. Effect of methanolic plant extracts on AGEs formation in the Glucose-BSA model in vitro

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According to these data, it can be concluded that the methanolic extract of *Tamarind indica* has a high antiglycation potency which might be attributed to its antioxidant activity. This result could lead to the characterization of the bioactive compounds which have the strong potential to be applied toward the prevention of glycation-associated complications in patients with diabetes.

Acknowledgements

The authors are grateful to the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education and Graduate School, Chiang Mai University, Chiang Mai, Thailand for the financial support.

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VITA

Name **Miss Khwanta Kaewnarin**

Date of birth **5 April 1982**

Place of birth **Uttaradit, Thailand**

Education

2005-2008: **M.Sc. Biotechnology,** Department of Biotechnology (Biochemistry & Biochemical Technology), Chiang Mai University

Thesis: *"Effects of Enzyme Inactivation by Microwave Pretreatment on the Quality of Dried Longan Pulp"*

Supervisor: Assoc. Prof. Dr. Nuansri Rakariyatham

2001-2004: **B.Sc. Biochemistry and Biochemical Technology,** Department of Chemistry, Faculty of Science, Chiang Mai University

Thesis: *"Prevention of Browning Reaction in Dried Longan Pulp by Using Plastic Films"*

Poster presentation

1. K. Kaewnarin and N. Rakariyatham (2011).Effects of Thai edible plants on lipofuscin and reactive oxygen species (ROS) formation in *Caenorhabditis elegans*. PERCH-CIC congress VII Chemistry Enviroment and Society (2011): Jotien Palmbeach Hotel and Resort, Pattaya, Chonburi. 4-7 May 2011.

Proceeding

1. K. Kaewnarin and N. Rakariyatham (2010). Screening for antioxidant and antiglycation properties of Thai indigenous plants (Proceeding). International Conference on Biotechnology for Health living: The 22 nd Annual meeting of the Thai society for Biotechnology (TSB2010). Prince of Songkla University, Trang campus, Thailand, 20-22 October 2010, 1148-1152.

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Publication

1. Khwanta Kaewnarin, Lalida Shank, Hataichanoke Niamsup and Nuansri Rakariyatham (2013), Antioxidant and antiglycation activities of some edible plants, *Chiang Mai Journal of Science*, 2014, 41(1), 1-14.
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3. Inhibitory Effects of Phenolic compounds in *Ocimum sanctum* extract on the α-glucosidase activity and the formation of advanced glycation end-products. Chiang Mai Journal of Science, *In process*.

Scholarship

2008-2010 The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education, Chiang Mai University, Thailand.

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