



Antioxidant and Antiglycation Activities of Some Edible and Medicinal Plants

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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% (IC₅₀) 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 IC₅₀ values of 110.4, 214.4 µg/mL and 243.3 µg/mL, respectively. The IC₅₀ 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

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- κ B (NF- κ B) 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

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 lyophilize.

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

	Common name	Extracted part
<i>Allium cepa</i>	Onion	Whole bulb
<i>Allium ascalonicum</i>	Shallot	Whole bulb
<i>Allium sativum</i>	Garlic	Whole bulb
<i>Gynura divaricata</i>	-	Leaves
<i>Gynemmainodorum</i>	-	Leaves
<i>Coccinia grandis</i>	ivy gourd	Leaves
<i>Gynostemma pentaphyllum</i>	jiaogulan	Leaves
<i>Coriandrum sativum</i>	coriander	Leaves
<i>Apium graveolens</i>	chinese celery	Leaves
<i>Eryngium foetidum</i>	false coriander	Leaves
<i>Centella asiatica</i> Urban	Asiatic pennywort	Leaves
<i>Cissus quadrangularis</i>	-	Stems
<i>Andrographis paniculata</i> Wallex Nees	king of bitter	Leaves
<i>Clitoria ternatea</i>	blue pea	Flowers
<i>Musa sapientum</i>	banana	Flowers
<i>Tamarindus indica</i>	tamarind	Young leaves
<i>Psidium guajava</i>	guava	Young leaves
<i>Mangifera indica</i>	mango	Young leaves
<i>Dimocarpus longan</i>	longan	Young leaves
<i>Punica granatum</i>	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 (Na₂CO₃), 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

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 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:

$$\text{DPPH radical scavenging effect (\%)} = [(A_0 - A_t)/A_0] \times 100$$

where A_0 represented the absorbance of the control solution and A_t 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 $\mu\text{g}/\text{mL}$ 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 $\mu\text{g}/\text{mL}$) 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 $\mu\text{g}/\text{mL}$) of the selected plant extracts. Aminoguanidine (AG) (10-100 $\mu\text{g}/\text{mL}$) 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.

2.7 Statistical Analysis

All experimental results were presented as means \pm 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 μ g 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 (μ g QE/g)	
	EA extract	ET extract	EA extract	ET extract
<i>A.cepa</i>	0.04 \pm 0.0	0.04 \pm 0.0	2.96 \pm 0.2	8.49 \pm 0.2
<i>A.ascalonicum</i>	0.04 \pm 0.0	0.10 \pm 0.0	3.82 \pm 1.0	6.16 \pm 0.2
<i>A.sativum</i>	ND	0.02 \pm 0.0	2.96 \pm 0.4	1.39 \pm 0.0
<i>G.divaricata</i>	0.16 \pm 0.0	0.40 \pm 0.0	48.3 \pm 6.5	78.6 \pm 7.2 ^d
<i>G.inodorum</i>	0.08 \pm 0.0	0.38 \pm 0.0	50.4 \pm 1.4	159 \pm 6.4 ^{b,c}
<i>C.grandis</i>	0.07 \pm 0.0	0.18 \pm 0.0	68.4 \pm 4.3	51.3 \pm 4.9
<i>G.pentaphyllum</i>	0.16 \pm 0.0	0.13 \pm 0.0	49.0 \pm 5.1	26.3 \pm 3.2
<i>C.sativum</i>	0.05 \pm 0.0	0.09 \pm 0.0	22.2 \pm 0.8	15.4 \pm 0.4
<i>A.graveolens</i>	0.03 \pm 0.0	0.14 \pm 0.0	27.8 \pm 1.4	40.5 \pm 0.7
<i>E.foetidum</i>	0.05 \pm 0.0	0.07 \pm 0.0	19.0 \pm 1.0	9.26 \pm 2.0

Table 2. (Continue)

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

- Values are expressed as means \pm SD.

- ^{a-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.

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

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* ($IC_{50} = 110 \mu\text{g/mL}$) had significantly stronger inhibitory activity than *M.indica* and *P.guajava* extract ($IC_{50} = 214 \mu\text{g/mL}$ and $243 \mu\text{g/mL}$), respectively ($p < 0.05$). However, these extracts were found to be less effective than aminoguanidine ($IC_{50} = 50.2 \mu\text{g/mL}$) 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-deoxyglucosone, 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 ($54.1 \mu\text{g/mL}$) had statistically higher antiglycation activity than *P.granatum* and *D.longan* extract ($69.1 \mu\text{g/mL}$ and $74.2 \mu\text{g/mL}$, 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 ($IC_{50} = 91.2 \mu\text{g/mL}$) ($p < 0.05$). 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.

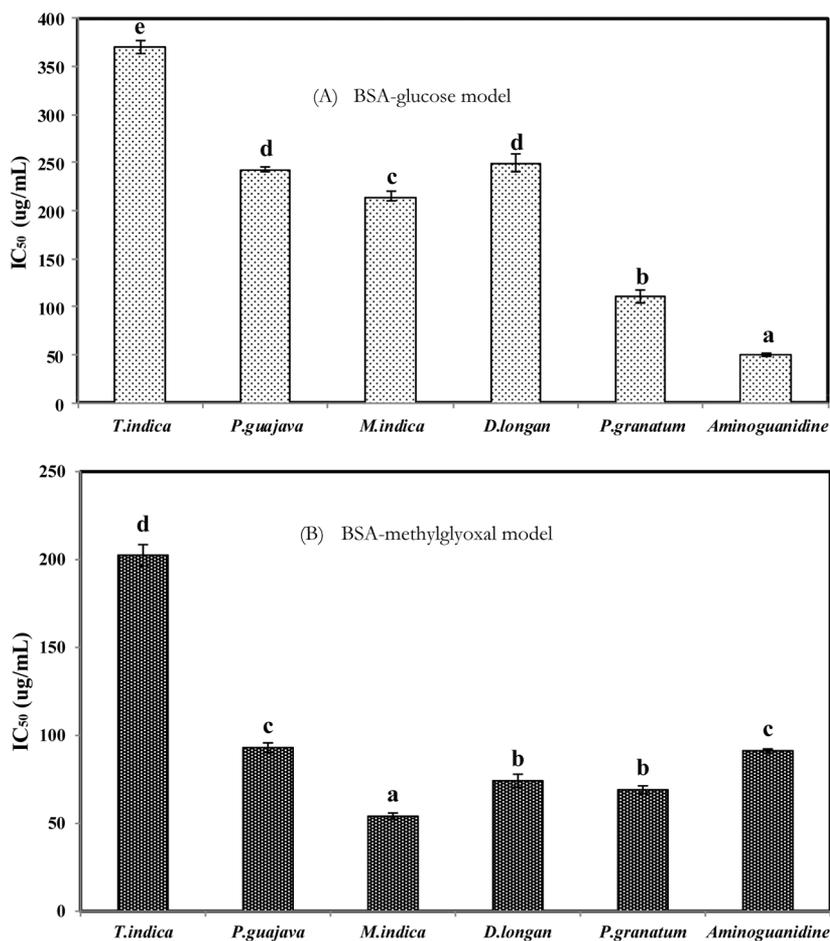


Figure 1. Inhibitory effect of the selected plant extracts (A) on the formation of glycation in BSA- glucose model (B) on the formation of glycation in BSA- methylglyoxal model. Aminoguanidine was used as a positive control. Different superscripts indicate statistically significant differences ($p <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

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 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.

Besides, Wu [24] has reported that flavonoids, especially, luteolin and rutin, developed a more significant inhibitory effect on methylglyoxal-mediated 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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