CHAPTER 1

Introduction

1.1 Rational and significance of problems

Prolonged hyperglycemia plays an important role in the development of diabetic complications leading to morbidity and mortality in diabetic patients. Several acute complications of diabetes can cause lipid and protein metabolic alterations in the body and more chronic irreversible complications (Brownlee, 2001; Lunceford and Gugliucci, 2005), such as retinopathy, cataracts, atherosclerosis, neuropathy and aging (Gugliucci, 2000; Pashikanti *et al*., 2010).The glycation of protein is one of the main primary mechanisms in the progression of diabetic complications. The accelerated AGE accumulation on extracellular proteins, such as albumin and low-density lipoproteins (LDL), induces protein dysfunction and tissue damage that is conductive to the vital factors for atherosclerosis and vascular basal membrane atrophy and the elevation of the blood pressure (Hsieh *et al*., 2007; Giugliano *et al.,* 1996). Furthermore, AGEs also form on intracellular proteins, such as histone, which is a composition of the DNA structure that results in genetic instability. Sugars other than glucose are also important in inducing protein glycation. In particular, pentoses, such as D-ribose, can participate in protein glycation much more than glucose. The highly reactive carbonyl species (RCS), glyoxal and methylglyoxal, were also efficient inducers for AGE formation. These compounds are up to 20,000 times more reactive than glucose (Thornalley, 2003; Pashikanti *et al*., 2011). The elevated AGE formation leads to a speed up in diabetic complications and modifications of proteins, lipids and DNA (Golej *et al.,* 1998; Miyata *et al.,* 1998).

Therefore, the inhibition of AGE formation is one of the therapeutic approaches in the prevention of diabetic complications. Currently, the stronger AGE inhibitor is aminoguanidine (AG), a hydrazine derivative synthetic inhibitor that showed the effective suppression of diabetic complications. Unfortunately, it proved to be toxic in long-term administration (Ou and Wolf, 1993; Freedman *et al.,* 1999; Okuda *et al.,* 1998). Therefore, efforts have been made to search for other safe and effective inhibitors against protein glycation.

Another therapeutic approach for the prevention of diabetic complications is to decrease postprandial hyperglycemia by retarding the absorption of glucose. Inhibition of α-glucosidase, which is a carbohydrate-hydrolyzing enzyme in the small intestine, has been considered. Recent attention has been focused on the bioactive compounds derived from natural plants having both antiglycation and α-glucosidase inhibitory properties. Several phytochemical investigations of the inhibitory effects on AGE formation have been reported. Procyanidins from cinnamon was shown to work effectively against protein glycation (Peng *et al.,* 2008). Flavan-3-ols from green tea exhibited the ability to scavenge carbonyl compounds by trapping (Lo *et al.,* 2006). Rutin and its metabolites showed inhibitory potential for non-oxidative AGE generation *via* BSA-glycation (Pashikanti *et al*., 2010). In previous studies, our teams have investigated several Thai edible plants which contain large amounts of bioactive compounds, particularly phenolic compounds that exhibited strong antioxidant activities (Chenwitheesuk *et al.*, 2005: Thitilertdecha *et al.*, 2008).

However, their phytochemical data of compounds involved in alleviating or preventing diabetic complications are scarce. For these reasons, the potential of Thai culinary plants has attracted vast amounts of interest in the prevention of diabetic complications.

Numerous phenolic compounds from the Lamiaceae family have been isolated and identified as being physiologicaly active natural compounds. *Ocimum sanctum*, a popular culinary plant of the Lamiaceae family, is considered one of the better known medicinal plants in Thailand. Its leaf has been traditionally used to treat a high blood pressure and to lower cholesterol. However, its bioactive constituents, which are

thought to be beneficial in antidiabetic treatment and the prevention of diabetic complications, are little known.

1.2 Formation of advanced glycation end-products (AGEs)

Advanced glycation end-products (AGEs) are modifications of proteins, lipids or DNA with reducing sugars (Goldin *et al.*, 2006) achieved through oxidation and glycation processes. AGEs form *in vivo* in hyperglycemic environments and during aging. They have been of widespread concern because they form adducts with various biological substances in both extracellular and intracellular proteins leading to the pathophysiology of diabetic complications.

1.2.1 Formation of exogenous AGEs

The exogenous sources of AGE are formed by carbohydrate auto-oxidation and degradation, Maillard reaction and lipid peroxidation which occur during thermal food processing including heating, sterilization or the use of a microwave oven. Researchers have reported that AGEs are found in foods cooked at extremely high temperatures (Uribarri *et al.*, 2007).

1.2.2 Formation of endogenous AGEs

1.2.2.1 Enzymatic AGE formation

The enzymatic pathways represent a series of reactions which are catalyzed by three kinds of enzymes: methylglyoxal synthase, amino oxidase(s) and cytochrome P450 IIE1 isozyme(s). Methylglyoxal synthase catalyzes the transformation of dihydroxyacetone-phosphate (DHA-P) into MGO. The catabolism of proteins through aminoacetone mediated with amine oxidase also produces MGO (Lyles and Chalmers, 1992). Enzymatic oxidations of ketone bodies (acetoacetate and acetone) induce the formation of MGO *via* the catabolism of acetol by cytochrome P450 IIE1 isozyme (s) (Casazza *et al.*, 1984). Finally, the MGO reacts with proteins during the middle stage of glycation and produce the advanced glycation end-products during the late stage of glycation.

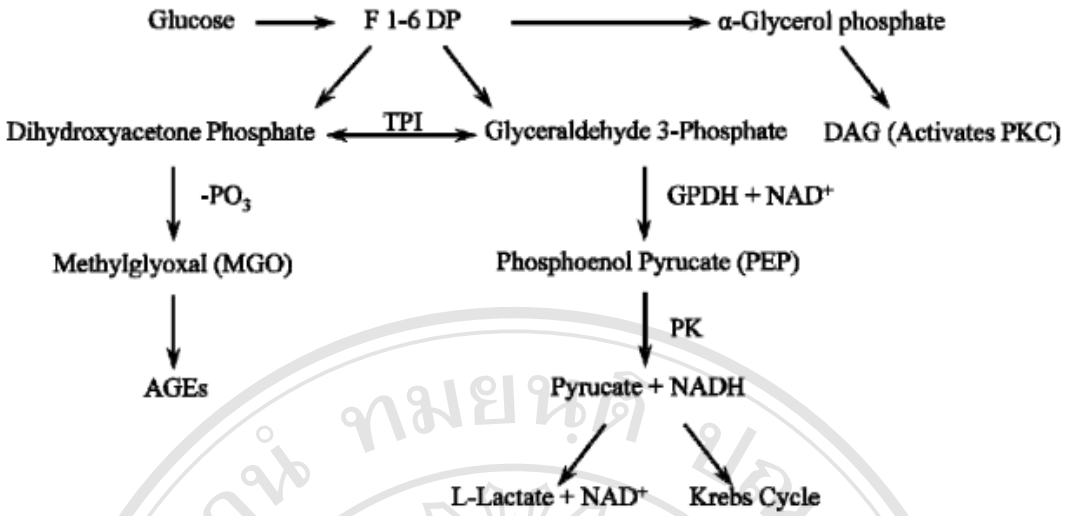


Figure 1.1 Pathways for producing methylglyoxal. F1-6 DP, fructose 1,6-diphosphate; TPI, triose phosphate isomerase; DAG, diacylglycerol; KPC, protein kinase C; PK, pyruvate kinase; LDH, lactate dehydrogenase; FN3K, fructosamine 3-kinase (Lyles and Chalmers, 1992)

1.2.2.2 non-enzymatic AGE formation

Non-enzymatic protein glycation, a spontaneous reaction between a free amino acid of protein or lipid and a carbonyl group of a reducing sugar, can be classified into three stages (Wu and Yen, 2005) as shown in Figure 1.2. The initial stage of protein glycation is formed when glucose reacts with an amino acid from the protein as a free reversible Schiff base. The Schiff base rearranges itself and attaches to more stable compounds called Amadori products, such as FL (N^{ε}-fructosyl-lysins) (Rabbani and Thornalley, 2008) and glycosylated hemoglobin A_{1c} (HbA1c); the most fundamental index derived from the blood of diabetic patients (Matsuura *et al*., 2002).

In the middle-stage of glycation, the subsequent rearrangement of Amadori products leads to the formation of di-carbonyl compounds, such as oxoaldehyde, methylglyoxal (MGO) and glyoxal (Monnier, 2003). Both methylglyoxal and glyoxal compounds can form crosslinks with two lysine residues thus becoming methylglyoxal-lysine dimer (MOLD) and glyoxal-lysine dimer (GOLD), respectively, which are found to be elevated in diabetic plasma proteins and cataractous lenses. Oxidation reactions are also involved in this process and are catalyzed by transition

metals while the generated superoxide radical ($O_2^{\bullet-}$) can be converted to a hydroxyl radical ($OH^{\bullet}$) (Hunt *et al.*, 1993).

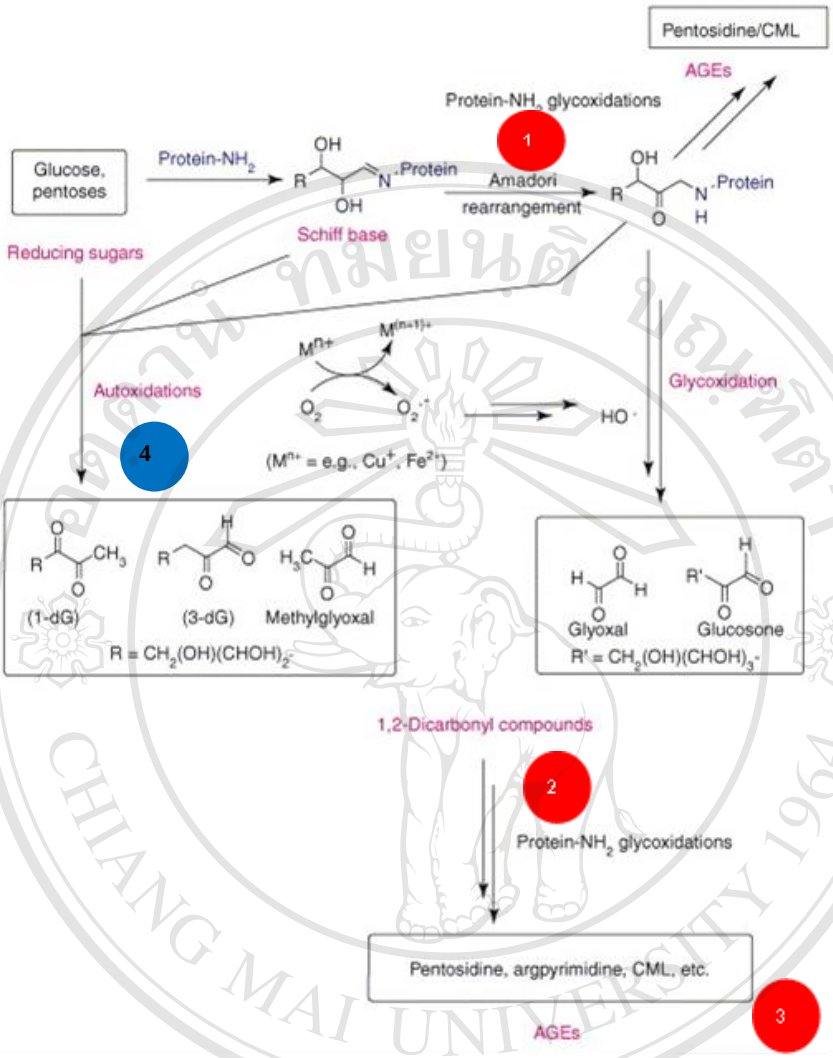


Figure 1.2 Schematic representations of the formation of advanced glycation end-products (AGEs) and AGE inhibitor's modes of action. (1) early stage; (2) middle stage, which involve carbonyl trapping agents; (3) late stage including crosslink breakers; (4) metal-ion chelators which suppress glyoxidation reactions (Reddy and Beyaz, 2006)

In the late-stage of glycation, these carbonyls then react with the amino group of proteins, leading to the formation of AGEs, including fluorescent cross-linking AGEs (e.g. pentosidine) and non-fluorescent AGEs (e.g. carboxymethyl-lysine

(CML)) (Hamelin *et al.*, 2003) through a serial cascade of complex reactions, including dehydration, oxidation, fragmentation and/or condensation (Ho *et al*., 2010). The AGE accumulation is believed to participate in many phases of the pathogenesis of micro vascular activity, such as nephropathy, retinopathy, atherosclerosis, neuropathy and cataracts in aged and diabetic patients (Ahmed *et al*., 2007).

Sugars other than glucose are also considered main factor for inducing protein glycation (Litchfield *et al.*, 1999). Generally, all reducing sugars can participate in protein gylcation reactions. Figure 1.3 shows an example of a reaction of each reducing sugar with protein. Glucose is assumed to be a key source of glycation in *vivo* under hyperglycemia conditions (Jacobson *et al*., 2001); (Pashikanti *et al*., 2010). However, glucose is only a weak glycation precursor and can occur over a month to a year, but only under oxidative conditions. Unlike glucose, pentoses, such as D-ribose or ADP-ribose, are more reactive in the glycation of protein than hexoses, because pentoses dissociate more readily to the open carbonyl form than hexoses (Pashikanti *et al*., 2011). It was shown that ADP-ribose is the most potent non-oxidative inducer of AGEs when histone H1 is the target protein of glycation (Cervantes-Laurean, *et al*., 1996), while D-ribose actively participates in protein glycation with both intracellular and extracellular proteins. The highly RCS glyoxal and methylglyoxal (MGO) compounds were implicated as sources for non-oxidative protein glycation. They were much more efficient inducers for the formation of AGE non-fluorescence, AGE fluorescence and cross-link proteins. Wei and colleagues (2012) have reported the glycating ability of different reducing sugars in the following order: D-glucose<D-mannose<D-galactose<D-xylose<D-fructose<D-arabinose<D-ribose<2-deoxy-D-ribose.

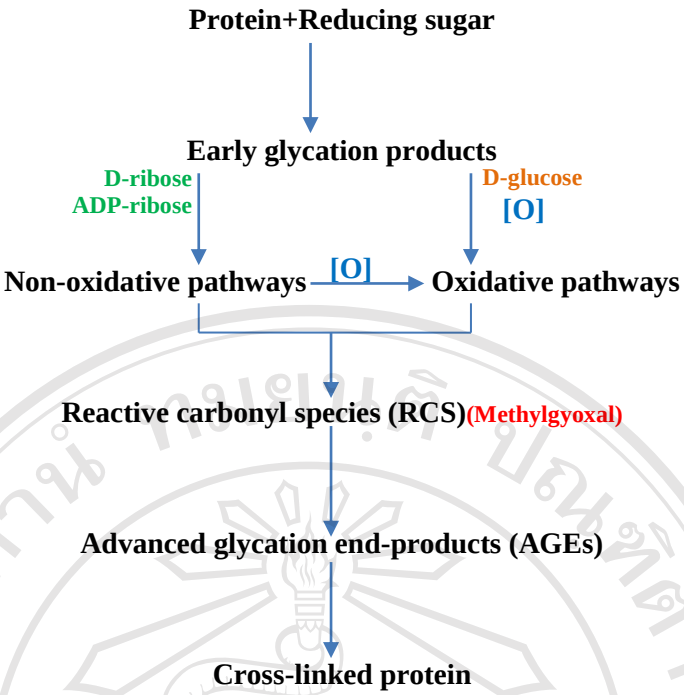


Figure 1.3 Pathway of the reaction of each reducing sugar participate in protein glycation

1.2.3 Type of AGEs structure

During the late stage of glycation, the highly reactive compounds react with an amino group of proteins to generate a variety of AGEs. On the basis of chemical structure, AGEs can be classified into 2 types depending on their fluorescence properties and crosslinking structure.

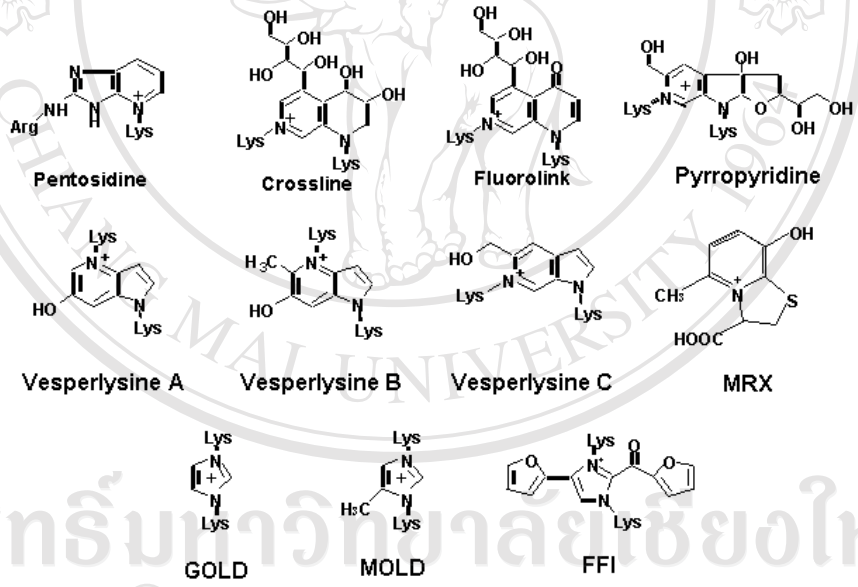
1.2.3.1 Fluorescent AGE crosslinking structures

The di-carbonyl intermediate compounds react with an amino acid residue during the late stage of glycation resulting in protein-protein cross-linked structures and fluorescent chromophores (Wu *et al.*, 2011): (A) fluorescent and crosslinking AGEs, such as crossline, 2-(2-furoyl)-4(5)-(2-furanyl)-1*H*-imidazole (FFI), glyoxal-lysine dimer (GOLD), methylglyoxal-dimer (MOLD), fluorolink, pentosidine and vesperlysines (Figure 1.4(A)).

1.2.3.2 Non-Fluorescent AGE non-crosslinking structures

Non-fluorescent AGEs structures are also generated initially by the same reaction of di-carbonyl intermediate compounds. However, these structures are formed based on glyoxidation more than the protein-protein cross-linked structures. One of the most abundant non-fluorescent AGE structures in the protein tissue is the N^{ε}-(carboxymethyl) lysine (CML), Figure 1.4 (B). The CML accumulation is frequently found under chronic hyperglycemia conditions. In the event of renal failure, the presence of CML in the vascular tissue causes tissue damage *(*Kislinger *et al.*, 1999) and the increased CML in the vascular permeability can enhance the risk of atherosclerosis (Wautier *et al.*, 2003).

(A) Fluorescent and crosslinking AGEs



(B) Non-fluorescent and non-crosslinking AGEs

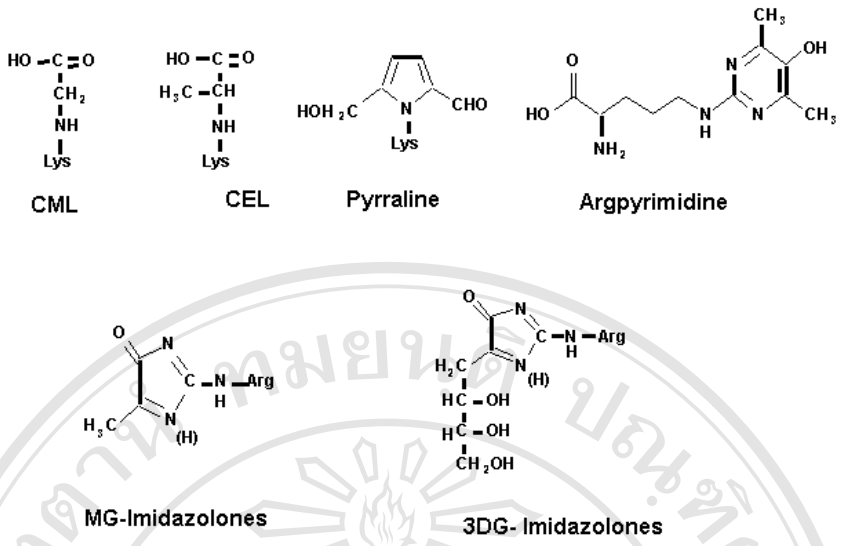


Figure 1.4 Chemical structures of two types of AGEs: (A) fluorescent and crosslinking AGEs; (B) non-fluorescent and non-crosslinking AGEs (Wu *et al.*, 2011).

1.2.4 Advanced glycation end-products (AGEs) effects on different organs in the body

Advanced glycation irreversibly and progressively modifies proteins, generating the adavanced glycation end-products (AGEs). Therefore, these AGEs play an important role in the structural and functional alteration of the various proteins in many vital organs. Consequently, the accelerated AGE accumulation leads to the progression of several diabetic complications, such as cardiovascular disease, retinopathy, Nephropathy, Neuropathy and DNA dysfunction (Figure 1.5).

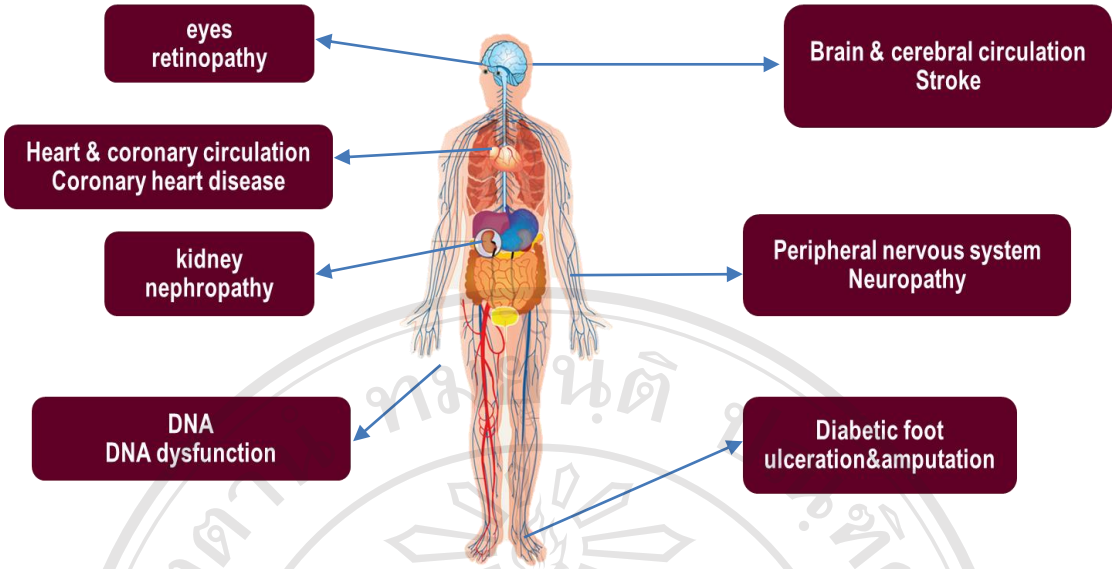


Figure 1.5 Advanced glycation end-products in the development of diabetic complications

1.2.4.1 Cardiovascular disease

The effects of AGEs on the cardiovascular system involve the crosslinking of extracellular matrix proteins, such as albumin, low density lipoproteins (LDL), immunoglobulin (IgG), vitronectin and laminin through AGE-AGE intermolecular covalent bonds, or crosslinking. The crosslinking of extracellular matrix proteins can cause damage to the normality and flexibility of the matrix proteins resulting in vascular that induces diastolic heart failure (Hartog *et al.*, 2007). Formation of AGEs on laminin, a key structural protein in the basement membrane, leads to a reduced binding to type IV collagen, reduced polymer elongation, and reduced binding of heparin sulfate proteoglycan; the key factors impairing the leaking of plasma proteins. Serum albumin comprises about 60% of human plasma proteins. It is highly sensitive to glycation (Wei *et al.*, 2012).The glycated LDL promotes plaque destabilization in the vascular system leading to atherosclerosis (Goh and Cooper, 2008). Furthermore, the crosslinking of collagen is considered an important factor in the development of diabetic cardiomyopathy and arterial disease.

1.2.4.2 Retinopathy

Glycation of eye lens protein has been considered one of the mechanisms which is responsible for both aged-related and diabetic cataracts leading to blindness (Muthenna *et al*., 2011). In addition, the collagen in the eye lens proteins contain higher hydroxylysine residue which can readily generate glycoprotein. Previous studies have reported that the formation of AGE *in vivo* contributes to cataracts by altering the surface charge of the protein, leading to change in conformation and decreased transparency of the eye lens protein (Beswick and Harding, 1987; Kumar *et al.*, 2004; Luthra and Balasubramanian, 1993).

1.2.4.3 Nephropathy

Kidneys play an important role in the metabolism of AGEs. Renal proximal tubule cells absorb and catabolize AGEs from the glomerular filtration (Gugliucci and Bendayan, 1996). In diabetic patients, the elevation of AGEs, as a result of the reduced renal metabolism of AGEs, can impair the function of the cells leading to uremic complications, including dialysis-related amyloidosis (Miyata *et al.*, 1998). Furthermore, the affinity binding between AGE receptors (RAGE) and AGEs causes expression of vascular endothelial growth factors and activation of inflammatory cells in the diabetic glomerulus, leading to albuminuria and glomeruloscelosis (Saito *et al.*, 2005; Wendt *et al.*, 2003).

1.2.4.4 Neuropathy

The AGEs have been shown to accumulate in the myelin and tubulin of peripheral nerves (Nawale *et al*., 2006). An amyloid protein can react with reducing sugars to form AGE formation and/or react with the reactive carbonyl species to promote the crosslinking of the proteins. The cross-linked proteins result in a loss of the protein conformation and are converted into protein aggregates with a predominant β-sheet form (Gella and Durany, 2009). The protein aggregation of amyloid plaques is one of the major causes of many neurodegenerative diseases, such as Alzheimer's disease (Christen, 2000). Like any other proteins, the AGE formation of the amyloid

protein can bind with the RAGE. The affinity binding can induce cytokine expression, resulting in adverse cytotoxic pro-inflammatory effects (Cameron *et al.*, 2005).

1.2.4.5 DNA dysfunction

Several studies have established that protein and DNA are readily glycated *in vivo* and *in vitro* to form DNA-advanced glycation end-products AGEs (DNA-AGEs) and protein advanced glycation end-products AGEs (protein-AGEs) (Akhter *et al.*, 2013). It has been shown that DNA and nucleotides can be efficient glycation targets (Seidel and Pischetstrider, 1998). *In vitro* nucleobases and double strains DNA (dsDNA) react with sugars in a similar way as proteins do in causing the formation of DNA-AGEs at the late stages of glycation (Knerr and Severin, 1993). DNA glycation results in the alteration of the DNA structure, *viz.* depurination, single strand breaks and mutations, such as insertions, deletions and transposition (Ahmed *et al.*, 2011). The DNA-AGEs leads to the loss of genomic integrity and partial unwinding and/or fragmentation of the double helix. In this regard, ribose and ADP-ribose can participate in glycation reactions. Ribose, which is a naturally occurring pentose monosaccharide in all living cells and a key component of many important bio-molecules, such as riboflavin, RNA and ATP (Akhter *et al.*, 2013), is the most reactive inducer in protein glycation (Khalifah *et al.*, 1996).

1.3 Methodologies for measurement of advanced glycation end-products (AGEs)

1.3.1 Fluorescence spectrometry

Due to the fact that most AGEs showed characteristic fluorescence with an excitation maximum at approximately 370 nm and an emission at 440 nm (Figure 1.6), detection of AGE formation through fluorescent spectrophotometry is a widely available method (de la Maza *et al.*, 2012). The fluorometric assay is inexpensive and relatively simple to execute.

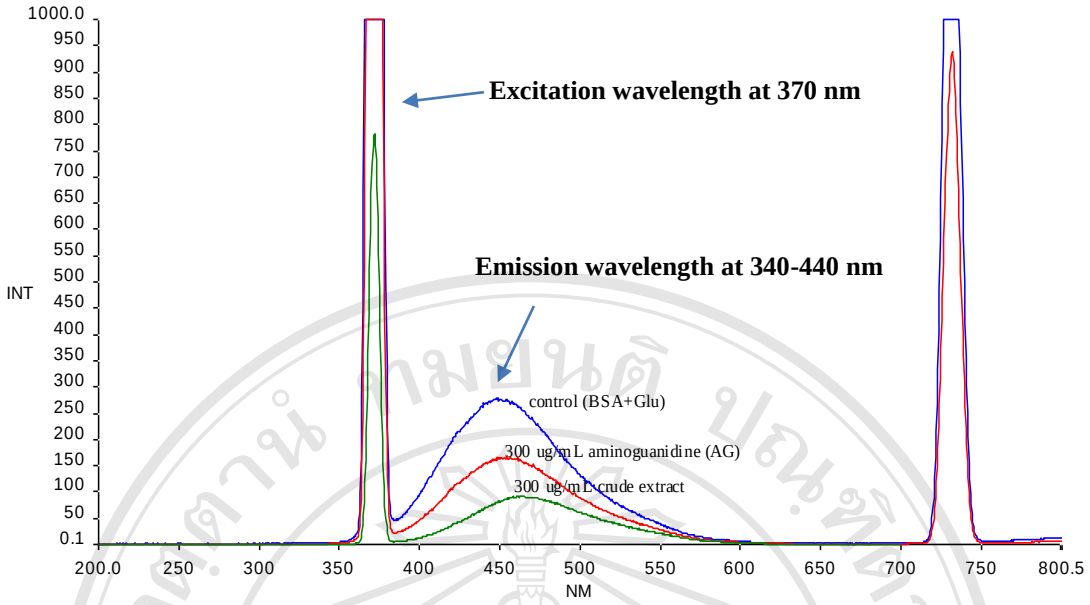


Figure 1.6 Fluorescence spectra of advanced glycation end-products (AGEs) measured by fluorescence spectrometry.

Vashishth (2009) has demonstrated that the fluorometric assay was able to measure the different wavelength of the fluorescent crosslinking of collagen (Figure 1.7). Excitation/emission wavelengths for the AGE crosslinks were pentosidine (335/385 nm), CEL/CML (340/455 nm), crossline (379/463 nm), vesperlysines A and B (366/442 nm) and vesperlysine C (345/405 nm). However, the limitation of this assay is not a precise identification of the specific AGE crosslinks. Moreover, the non-fluorescent AGE crosslinks could not be measured by this assay.

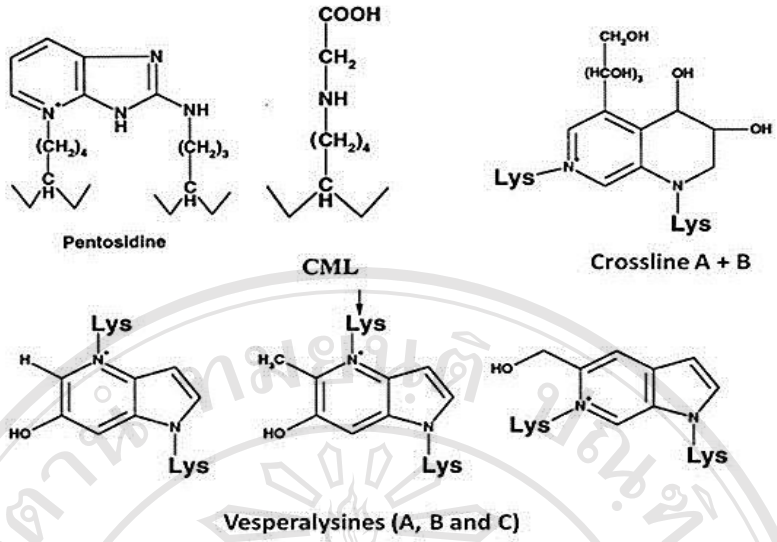


Figure 1.7 AGE crosslinks of collagen were measured using the fluorometric assay (Vashishth, 2009)

In 1997, Wrőbel *et al*. developed the flow injection assay (FIA), which employs on-line spectrophotometric and spectro fluorimetric detection, using the flow system of the HPLC equipment. This tool was used to detect microvascular complications in patients with type 2 diabetes.

1.3.2 High performance liquid chromatography (HPLC)

1.3.2.1 Direct methyl glyoxal (MGO) trapping scavenging by HPLC technique

HPLC methods are also used for the quantification of methylglyoxal (MGO), which is an intermediate of the middle stage of protein glycation. However, MGO can not be directly detected. It has to react with the derivatization agent, such as *O*-phenylenediamine (OPD) to form quinoxaline adducts (Figure 1.8). These quinoxaline adducts can readily be measured either by UV detector at 300 to 360 nm or by fluorescent detector with an excitation wavelength range of 300 to 360 nm and an emission wavelength range of 380 to 450 nm (Peng *et al*., 2008).

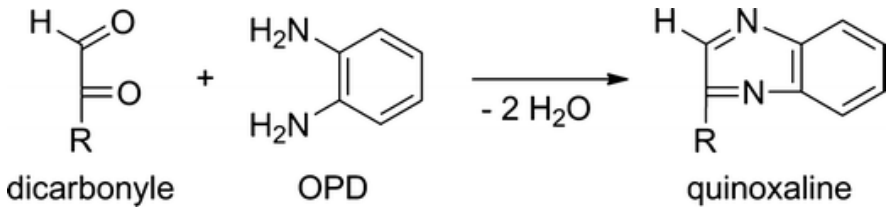


Figure1.8 Derivatization reaction of α-dicarbonyl compounds with *o*-phenylenediamine (OPD) to reveal corresponding quinoxalines (Mittelmaier *et al*., 2011)

Recently, the liquid chromatography/ electrospray ionization/ mass spectrometry (HPLC-ESI-MS^n) method has been developed to identify the trapping that occurs between catechins and carbonyl compounds (Wang *et al.*, 2011). The results have shown that carbonyl compounds (glyoxal) react with catechins into five adducts.

1.3.2.2 Identification and quantification of AGEs by LC-MS technique

To date, numerous AGEs have been identified and quantified by LC-MS. This technique has been applied to investigate skin-aging (Gkogkolou and Bőhm, 2012). Both Fluorescent and non-fluorescent AGE products, such as carboxymethyl-lysine (CML), pentosidine, carboxyethyl-lysine (CEL), fructose-lysine, glyoxal-lysine dimer (GOLD), and methylglyoxal-lysine dimer (MOLD) are the key biomarkers in skin- aging that could be detected by LC-MS (Table 1.1).

Table 1.1 Detection of AGEs in skin (Gkogkolou and Bőhm, 2012)

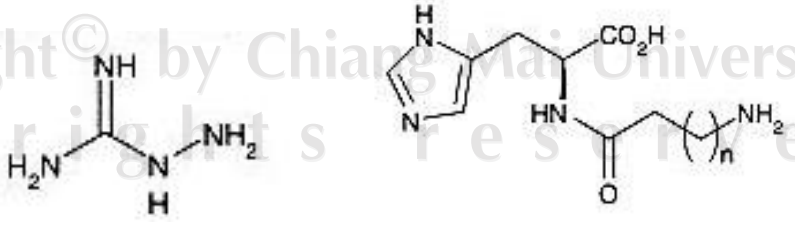
AGEs	**Skin compartment involved**	**Targets of glycation**	**Method of detection**
carboxymethyl-lysine (CML)	Epidermis	Epidermis	LC-ESI-TOF-MS
Pentosidine	Aged and diabetic dermis	collagen	Reversed-HPLC
glyoxal-lysine dimer (GOLD)	Aged dermis	collagen	LC-MS
methylglyoxal-lysine dimer (MOLD)	Aged dermis	collagen	LC-MS
carboxyethyl-lysine (CEL)	Aged dermis	collagen	LC-MS

1.3.3 Polyacrylamide gel electrophoresis (SDS-PAGE)

Protein gel electrophoresis is used to separate protein samples under denaturing conditions and to purify specific components of a mixture that contains more than one protein. The charge to the mass ratio of each protein determines its migration rate through the gel. A negative charge is provided by the inclusion of sodium dodecyl sulfate (SDS) in the loading gel and electrophoresis buffers. Gel electrophoresis of glycated protein mixtures can provide information about crosslinked polymerization of proteins during incubation. The crosslinked proteins are larger and have lower electrophoretic mobility than non-crosslinked protein species. In the presence of SDS, the average molecular weight of cross-linked proteins can be estimated. Depending on the pore size of the gel matrix, the degree of crosslinking, such as dimers and trimers can be identified. The absence of SDS-PAGE can be used to separate polymerized glycated proteins depending on their charge properties. The glycated protein migrates faster to the anode than the native protein due to the attachment of sugar residues to the amino groups of proteins.

1.4 The inhibition of AGE accumulation

In addition, AGE accumulation *in vivo* has been implicated as a major factor in the pathogenesis of diabetic complications. Thus, the AGE inhibition strategies have focused on delaying and preventing the progression of diabetes and diabetic complications. Several synthetic compounds, such as aminoguanidine (AG) and carnosine (Figure 1.9), have been developed to reduce the AGE accumulation in clinical trials. However, the development and investigation of AGE inhibitors from natural sources are alternative approaches for preventing diabetic complications.



Aminoguanidine **Carnosine**

Figure 1.9 Representative advanced glycation end-products (AGEs) inhibitors (Reddy and Beyaz, 2006).

1.4.1 Aminoguanidine

Aminoguanidine (Pimagedine®), the first synthetic AGE inhibitor, is a nucleophilic hydrazine compound. It prevents AGE formation by blocking carbonyl intermediates during the middle stage of glycation and glucose auto-oxidation (Figure 1.10) (Ahmed, 2005). Moreover, Aminoguanidine (AG) may act as an antioxidant through the attenuation of oxidative stress (Bolton *et al.*, 2004). In diabetic nephropathy in animal studies, aminoguanidine reduces mesangial expansion and the thickening of the basement membrane (Ellis and Good, 1991) and collagen-linked fluorescence and cross-linking (Soulis *et al.*, 1996). In the latter study, Placebo-controlled clinical trials have been conducted with aminoguanidine in types 1 and 2 diabetic patients. However, the use of aminoguanidine is not being further advanced due to adverse side effects (Thornalley, 2003). These side effects were clearly observed in Phase III clinical trails in diabetic patients and included flu-like symptoms, gastrointestinal disturbances and anemia (Whittier *et al.*, 1999). Some of the adverse side effects were attributed to vitamin B6 deficiency (Reddy and Beyaz, 2006).

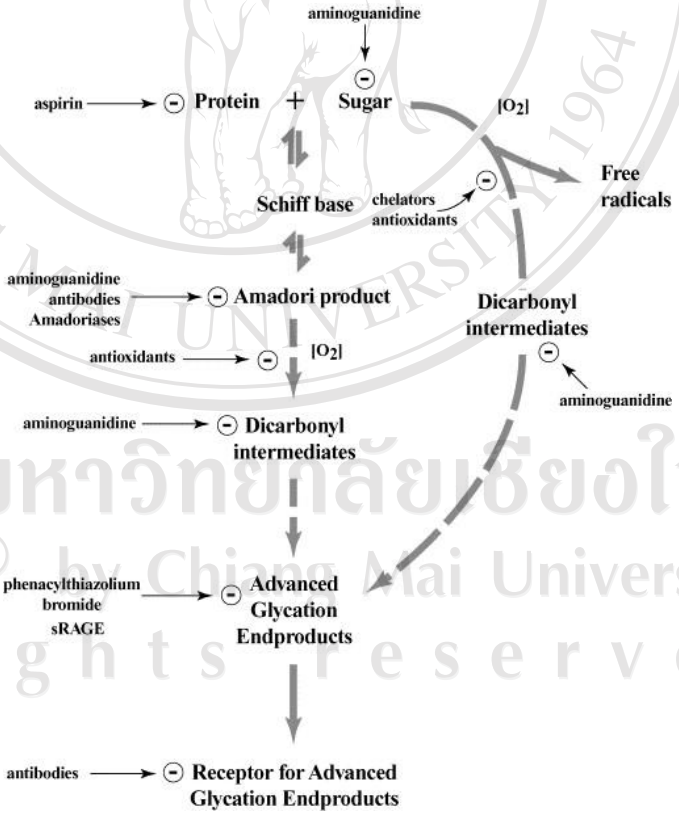


Figure 1.10 Schematic presentation of synthetic AGE inhibitors and their modes of action (Ahmed, 2005).

1.4.2 Carnosine

Carnosine (β-alanyl-L-histidine) is a commercially available drug. Its analogues are considered effective antioxidant properties due to their ability to chelate transition-metal ions. In addition, they also work as antiglycation agents. However, carnaosine is not comparable in terms of the AGE inhibitory action with aminoguanidine. Previous studies have reported that carnosine could protect against α-crystallin, superoxide dismutase and catalase in both protein glycation and antioxidant activities (Hipkiss *et al*., 1995). *N*-acetylcarnosine, a produg for carnosine, is useful for age-related cataract management and prevention (Babizhayev *et al*., 2004). Recently, it has been reported that carnosine and homocarnosine might be responsible for the prevention of Alzheimer's disease by detoxifying the highly reactive aldehyde acrolein, a neurotoxic aldehydes in the brain (Carini *et al*., 2003).

1.4.3 Polyphenols

Polyphenols are the most abundant dietary antioxidants present as the common constituents of fruits, vegetables, cereals, seeds, nuts, chocolate and certain beverages, such as coffee, tea and wine. They have been shown to possess many health benefits, such as the prevention of cancer, neurodegenerative diseases, cardiovascular diseases and diabetes. Moreover, polyphenols, particularly flavonoids, are responsible for anti-glycation activity. Several studies have demonstrated that the antiglycation activity correlates significantly with the phenolic content of the tested plant extracts. Some of important phenolic compounds, such as phenolic acid, flavonoids and many others have been reported to possess antiglycation activity.

1.4.3.1 Phenolic acids

Phenolic acids are generally found in almost all vegetables and fruits. The chemical structure of the C1-C6 and C3-C6 backbones is based on its unique phenol ring. Phenolic compounds are widely found in nature, such as in caffeic acid, ferulic acid, rosmarinic acid and gallic acid (Figure 1.11). In 2009, Gugliucci *et al* reported that caffeic acid in *Ilex paraguariensis* extracts could inhibit the generation of fluorescent AGEs *in vitro*. Ferulic acid also showed the inhibition of carboxymethyl-

lysine (CML) and fluorescent AGEs *in vitro*. Silván *et al.* (2011) mentioned that ferulic acid might prevent AGE formation by acting as an antioxidant, binding amino groups and suppressing sugar auto-oxidation and early Maillard reaction products (MRPs) degradation. In 2011, Miroliaei *et al.* revealed that in the presence of rosmarinic acid, a dimeric structure of caffeic acid in *Melissa officinalis* L. extract, demonstrated the prevention of structural changes of BSA induced by D-glucose near to its native conformation. Also, Ma *et al.* (2011) reported that rosmarinic acid, isolated from *Salvia miltiorrhiza Bge*, has a more potent inhibitory effect against the AGE formation and α-glucosidase activity than aminoguanidine (a positive control). This could suggest that rosmarinic acid is responsible for the antiglycation activity. Gallic acid and its derivatives also displayed inhibitory activities on aldose reductase and on AGE formation in the BSA-glucose model system.

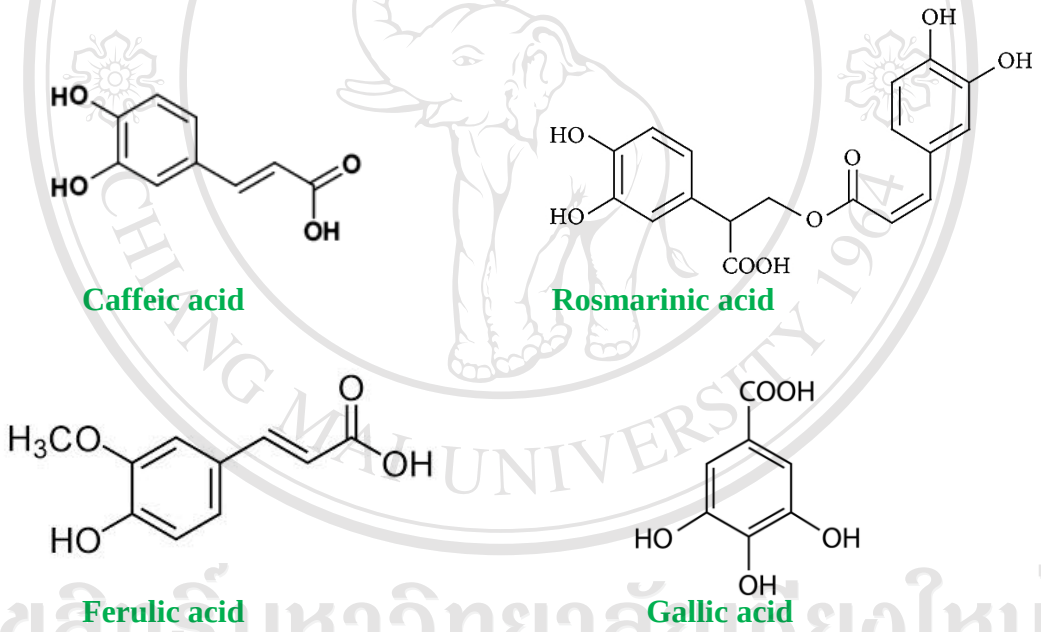


Figure 1.11 Chemical structures of phenolic acids

1.4.3.2 Flavonoids

Flavonoids have the C6-C3-C6 general structural backbone in which two C6 units are of the phenolic structure. Due to the hydroxylation pattern and variations in the chromane ring (Ring C), flavonoids can be divided into different sub-

groups, such as anthocyanins, flavan-3-ols, flavones, flavanones and flavonols (Figure1.12). These flavonoids are generally found in the plant kingdom.

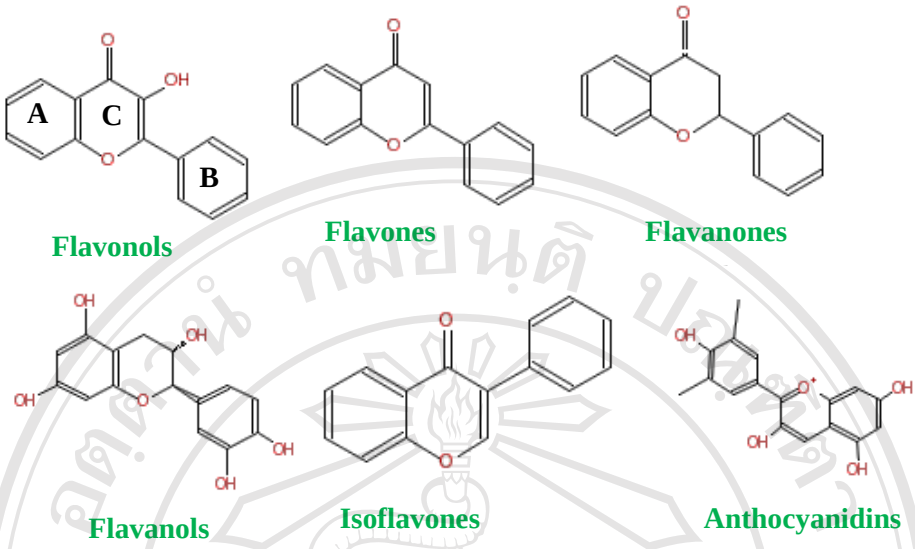


Figure 1.12 Structure of the major classes of flavonoids

Kaempferol, a well known antioxidant flavonol, was identified in the *Chrysamthemum* species which showed strong inhibition against AGE and CML formations in BSA/glucose (fructose) systems (Tsuji-Naito *et al*., 2009). In 2010, Kim *et al.* observed that aged rats that were administered with kaempferol over the short-term could modulate proinflammatory NF-κB activation through the suppression of AGE formation.

Quercetin is another flavonol, found in citrus fruit, buckwheat and onions, as well as berries and grapes. Several studies have reported on the ability of quercetin to against protein damage (AGE formation) when used in *in vitro* models (Soman *et al*., 2010; Wu and Yen, 2005; Wu *et al*., 2009b). Quercetin, gallic acid, ferulic acid and quercetin glycosides are also found in guava leave extracts. These phenolic compounds showed significantly decreased fasting blood glucose levels in streptozotocin-induced diabetic rats. They were observed to decrease glycation products and lipid peroxidation and improve the antioxidant status in a dose-dependent manner (Soman *et al*., 2010). Besides, the quercetin glycosides, isoquercetrin (quercetin-3-β-glucopyranoside) and hyperin (quercetin-3-D-galactoside), displayed remarkable

inhibitory activity against the AGE formation which was stronger than the aminoguanidine (AG) positive control (Lee *et al*., 2008). In 2011, Manaharan *et al.* reported that quercetin-3-O-β-D-galactopyranoside, which is the major bioactive compound in the ethanolic extract of *Peltophorum pterocarpum* leaves and bark showed more significant antiglycative activity and α-glucosidase inhibitory activity than acarbose, the commercial carbohydrate inhibitor.

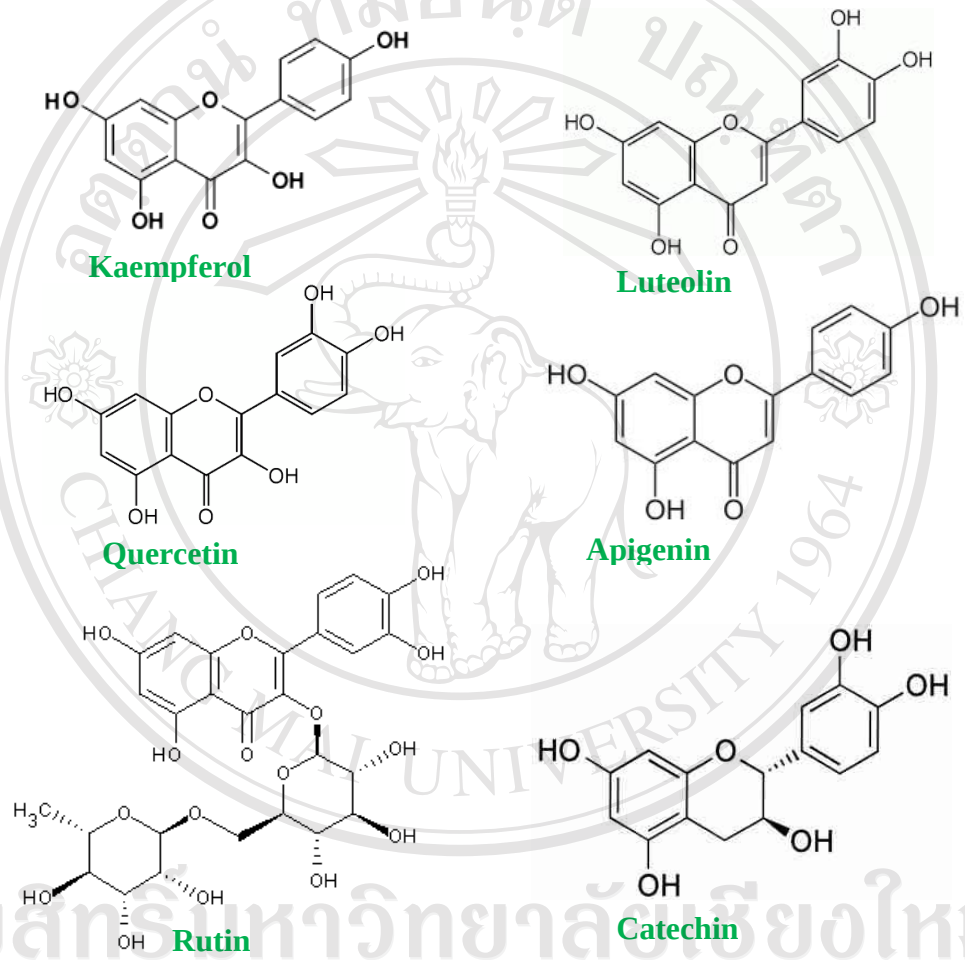


Figure 1.13 AGE inhibitors of flavonoids.

Quercetin-3-*O*-rutinoside (or what is referred to rutin) is found in fruits, vegetables and plant-derived beverages, such as tea and wine. Rutin metabolites, particularly 3,4-dihydroxyphenylactic acid (DHPAA) and 3,4-dihydorxytouluene (DHT), are effective inhibitors of the formation of N^{ε}-carboxymethyl-lysine (CML) and fluorescent derivatives (370-440 nm and 335-385 nm) in histone H1 induced by ADP-

ribose (Pashikanti *et al*., 2010). These rutin metabolites in plasma are expected to neutralize glyoxal and methylglyoxal in plasma concentrations. Rutin was also found to inhibit the formation of glycation products in collagen type I that was induced by glucose *in vitro* (Cervantes-Laurean *et al*., 2006) and to be an effective inhibitor of lipoprotein glycation by increasing the resistance of low-density-lipoprotein (LDL) under high glucose concentrations *in vitro* (Wu *et al*., 2009a).

Luteolin and apigenin are flavone aglycone compounds which are found in such foods as parsley, artichokes, basil, and celery. In 2005, Wu *et al.* reported on the inhibitory effects of naturally occurring flavonoids on different stages of protein glycation in *in vitro* model systems. Among the flavonoid compounds, luteolin, quercetin and rutin exhibited inhibitory activity on HbA_{1c} formation in the early stage of protein glycation, while in the middle stage, luteolin and rutin also showed significant inhibitory effects on methylglyoxal-medicated protein modification. In addition, luteolin was found to be a potent inhibitor of both the AGEs formation and the subsequent cross-linking of the proteins during the last stage of glycation. The presence of luteolin with maysin (a flavone glycoside) in corn silk played a role in suppressing the AGE formation (Farsi *et al.*, 2008). In 2009, Tsuji-Naito *et al.* demonstrated that apigenin (4´,5,7-trihydroxyflavone) in *Chrysanthemum morifolium* R. was the main component of the plant extract inhibiting the AGE accumulation, although it was a minor flavonoid aglycone in this plant extract. Apigenin, luteolin, apigenin-7-O-β-D-glucuronide methyl ester and apigenin-7-O-β-D-glucuronide were identified in the ethyl acetate soluble extract of the flower of *Erigeron annus* (Yoo *et al.*, 2008). They reported that apigenin-7-O-β-D-glucuronide methyl ester and apigenin-7-O-β-D-glucuronide showed significant inhibitory activities toward aldose reductase and AGE formation. Vitexin and isovitexin are flavone C-glucosides which have been identified in mung bean extracts (Peng *et al.*, 2008). Both vitexin and isovitexin showed significant inhibitory activities against the formation of AGEs induced by glucose or methylglyoxal, but they failed to directly trap reactive carbonyl species, such as methylglyoxal.

Catechin epicatechin, flavonol compounds found in green tea, are an excellent source of many polyphenol antioxidants (Babu *et al*., 2007). The most

important catechins of green tea are **(-)**-epicatechin (EC), (-)-epicatechin -3-gallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin -3-gallate (EGCG) and epicatechin-3-gallate (ECG) (McKay and Blumberg, 2002). In 2009, Rasheed *et al.* reported that EGCG significantly decreased AGE-stimulated gene expression and the production of TNFα and matrix metalloproteinase-13 (MMP-13) in human chondrocytes. EGCG has also been shown to prevent intracellular AGEs formation and the production of proinflammatory cytokines in monocytes under hyperglycemic conditions. Ho and colleagues (2010) demonstrated that EGCG is capable of trapping reactive dicarbonyl species, such as methylglyoxal (MGO) and glyoxal (GO) compounds. Catechin and epicatechin procyanidin B2 isolated from cinnamon bark was also proven to possess a significant MGO trapping activity, among which procyanidin B2 revealed the strongest AGE inhibition (Peng *et al.*, 2010; Peng *et al.*, 2008).

1.4.4 Other phenolic compounds

1.4.4.1 Stilbene glucoside

A stilbene glucoside (2,3,5,4´-tetrahydroxystilbene-2-*O*-β-glucoside) is a natural compound with strong antioxidative and anti-inflammatory properties, which has been reported as a major compound derived from *Polygonum multiflorum* Thumb. (a traditional Chinese herbal tea) (Lv *et al.*, 2007). It has been shown to effectively inhibit AGE formation through the trapping of reactive MGO under physiological conditions (pH 7.4, 37ºC) (Lv *et al.*, 2010).

1.4.4.2 Terpenes, essential oils, carotenoids and polyunsaturated fatty acids

A terpene is one of a class of unsaturated hydrocarbons which is found in the essential oils of many plants. 5,6-dehydrokawain (DK), dihydro-5,6-dehydrokawain (DDK) and 8(17), 12-Labdadiene-15, 16-dial (labdadiene) were isolated from the rhizome of *Alpinia zerumbet*. The results showed that labdadiene was a potent antiglycation agent against fructosamine adduct and α-dicarbonyl compound formation (Chompoo *et al.*, 2011). In 2010, Sun *et al*. reported that carotenoids, especially lutein in *Chlorella zofingiensis* and unsaturated fatty acids, mainly linoleic acid, arachidonic

acid and eicosapentaenoic acid in *Nitzschia laevis*, are responsible for the strong antiglycative capacities of these plants.

Carnosic acid, and carnosol are diterpene compounds which have been isolated from rosemary. They showed very effective antiglycation activities in different protein models (Hsieh *et al*., 2007).

1.4.5 Other antiglycative compounds

Vitamins are also a part of the human antioxidant defense system. For example, administration with vitamin E could prevent renal hypertrophy in streptozotocin diabetic rats (Kim *et al.*, 2000).Vitamin C and α-tocopherol have proven to be powerful advanced glycation end-products (AGEs) inhibitory agents, much more prominently effective than AG *in vitro* (Booth *et al.*, 1996; Vinson and Howard, 1996). Pyridoxamine (vitamin B6, Pyriodrin®) and thiamine pyrophosphate have the potential to be at least as effective at AGE formation as aminoguanidine (Booth *et al.*, 1997). Pyridoxamine, one of three natural forms of vitamin B_6, has been shown to inhibit the formation of CML and N^{ε}-(carboxyethyl) lysine (CEL), the non-fluorescent and non-crosslinking AGE structure. Pyridoxamine inhibits the AGE formation by trapping dicarbonyl intermediates (Metz *et al.*, 2003). In addition, it also traps reactive oxygen species (ROS) of the oxidative degradation of the Amadori intermediates in Maillard reactions (Voziyan and Hudson, 2005a). Although, it could trap both reactive carbonyl compounds and ROS, it is not efficient as an aminoguanidine for therapeutic purposes. Pyridoxamine is currently in Phase III clinical trials for the treatment of diabetic nephropathy (Voziyan and Hudson, 2005b; Williams, 2004).

1.5 Role of α-glucosidase in diabetes

One therapeutic approach in the treatment of diabetes is the inhibition of α-glucosidase leading to decrease post-prandial hyperglycemia. The inhibition of carbohydrate hydrolyzing enzymes, such as α-glucosidase and α-amylase in the digestive tract can retard and reduce the digestion and absorption of glucose in the blood circulation. Stabilization of blood glucose level prevents hyperglycemia and

diabetic complications. Prolong diabetic complications are the main cause of morbidity and mortality in diabetic patients (Brownlee, 2001).

α-Glucosidase is an exo-type carbohydrase generally found in microorganisms, as well as plant and animal tissues (Skeggs *et al*., 1956). α-Amylase catalyzes the initial step in the hydrolysis of starch to various small oligosaccharides consisting of maltose, maltotriose and a number of α-(1-6) and α-(1-4) oligoglucans. These products are further degraded by α-glucosidase to glucose which is absorbed and then enters to the blood circulation (Figure1.14). Degradation of dietary starch to glucose proceeds rapidly and leads to the increased post-prandial plasma glucose levels in hyperglycemia conditions.

Acarbose is currently used as α-glucosidase and α-amylase inhibitor, and also induces certain side effects, such as abdotension, bloating, flatulence and diarrhea (Chakrabarti and Rajagopalan, 2002). Previous studies have reported that the excessive inhibition of acarbose on pancreatic α-amylase resulted in the abnormal bacterial fermentation of undigested carbohydrates in the colon (Bischcoff, 1994). Therefore, natural α-glucosidase inhibitors from plants are of interest due to their effective inhibitors with minimal side effects. Some plants with the inhibitory activity against α-glucosidase enzymes have been summarized in Table 1.2.

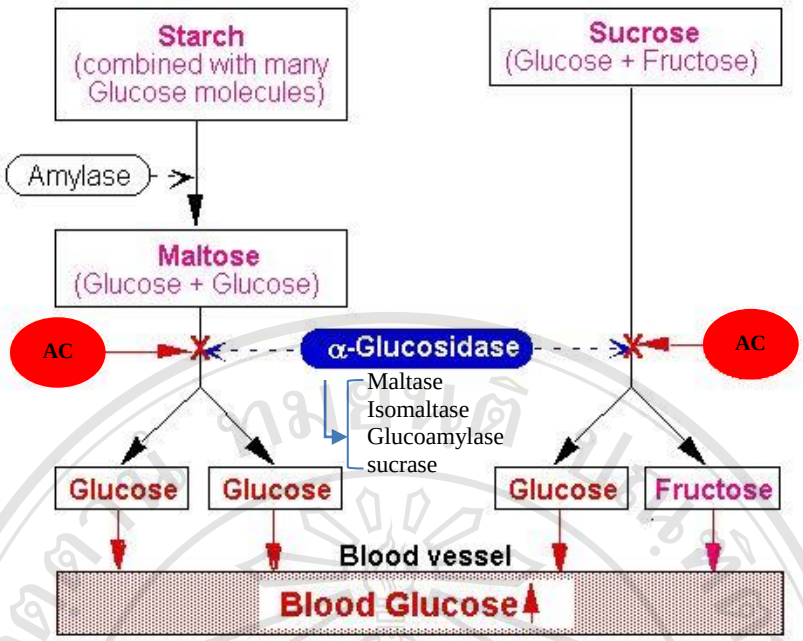


Figure 1.14 the mechanisms of α-amylase and α-glucosidase enzymes in the small intestine. Acarbose (AC), a standard α-glucosidase inhibitor, competitively inhibits the enzymatic hydrolysis of oligosaccharides

Table 1.2 plants with α-glucosidase inhibitory activity (Kumar *et al*., 2012)

Plant name	Part	Extract and active constituents	IC_{50}
Acosmiun panamense	Bark	Butanolic extract	109 µg/mL
Adhatoda vasica Nees	Leaves	Vasicine	125 µM
		Vasicinol	250 µM
Euclea undulata	Root bark	Acetone extract	49.95 µg/mL
Malmea depressa	Roots	Butanolic extract	21 µg/mL
Mangifera indica	Bark	Ethanolic extract	314 µg/mL
Morus alba	Leaves	1-deoxynojirimycin(s)	$7.7x10^{-5}$ mM
		1-deoxynojirimycin(m)	$1.7x10^{-4}$ mM
Penares schulzei	Bark	Schulzeines A	48-170 nM
		Schulzeines B	48-170 nM
		Schulzeines C	48-170 nM
Pine densiflora	Bark	(Pycnogenol)	5 µg/mL
Pine densiflora	Bark	Ethanolic extract	155 µg/mL
Syzygium malaccense	Bark	Casuarine 6-O-β-glucoside	5.7 µg/mL

1.6 Phytochemical data of plants used in the study of diabetes

There are many medicinal plants that have been suggested for use in the treatment of diabetes and diabetic complications. Thus, the daily consumption of dietary components, mainly derived from culinary plants which have reported antioxidant properties, is considered due to the phytochemical data on diabetic properties. The plant materials which were used in this study could be classified into 4 groups of (1) spices and condiments, (2) vegetables, (3) herbs and (4) fruits.

1.6.1 Spices and condiments

1.6.1.1 Family Lamiaceae: *Metha cordifolia* Opiz., *Ocimum sanctum*, *Ocimum basilicum* and *Ocimum americanum*

***Metha cordifolia* Opiz.**

***Ocimum sanctum* (white)**

Ocimum americanum

***Ocimum sanctum* (purple)**

Ocimum basilicum

Figure 1.15 The spices and condiment plants in the Lamiaceae family used in this study

Metha cordifolia Opiz. (kitchen mint), *Ocimum sanctum* (holy basil), *O. basilicum* (sweet basil) and *O. americanum* (hairy basil) belong to the Lamiaceae family are commonly used as spices for culinary purposes and have been used in traditional medicines in Thailand, especially the genus *Ocimum*. Several

pharmacological and clinical trials have been reported. Holy basil extract, which was obtained after boiling in water for 10 minutes, was able to maintain blood sugar levels in diabetic patients (Farnsworth and Bunyapraphatsara, 1992). In 1997, Rai *et al.* has revealed the hypoglycemic and hypolipidemic effects of *Ocimum sanctum leaves* in significantly reducing fasting blood glucose levels, uronic acid, total amino acid, total cholesterol, triglyceride and total lipid. The aqueous extract of *Ocimum sanctum* leaves significantly revealed a reduction in blood glucose level in both normal and alloxan-induced diabetic rats (Vats *et al*., 2002). Oral administration of the plant extract for 30 days led to a decrease in the plasma glucose level in streptozotocin induced diabetic rats (Vats and Yacav, 2004). Moreover, the antiasthemitic, antistress, antibacterial, antifungal, antiviral, antitumor, gastric antiulcer activity, antioxidant, antimutagenic and immunostimulant activities of this plants were also reported (Modak *et al*., 2007). Previous phytochemical investigations on *Ocimum sanctum* have described the isolation of two groups of active compounds, terpenoids and phenolic compounds. Over 25 terpenoid and fatty acid derivatives have been isolated from this plant (Gupta *et al*., 2007), such as eugenol and methyl eugenol as the main compounds in the essential oil of the *Ocimum* species (Cheng and Liu, 1983). These active compounds demonstrated anticancer, anti-HIV, antimicrobial and anti-inflammatory effects (Chattopadhyay *et al*., 1987). The phenolic compounds have been also identified from the leaves of *Ocimum sanctum*, which exhibited antioxidant and anti-inflammatory activities, including caffeic acid and its derivatives (rosmarinic acid), flavones (apigenin, luteolin, crisimarin, isothymusin, luteolin glycosides) (Devi *et al*., Pattanayak *et al*., 2010).

1.6.1.2 Family Alliaceae: *Allium ascalonicum* (shallot), *Allium cepa* (onion) and *Allium sartivum* (garlic)

Allium ascalonicum

Allium sartivum

Allium cepa

Figure 1.16 The plants of spices and condiments in the Alliaceae family used in this study

Shallots, onions and garlic are major components of many Asian diets which are widely believed to possess diverse biological activities. Numerous studies have focused on the hypoglycemic effect. Jain and Vyas (1975) have been shown the hypoglycemic effect of garlic extracts with different organic solvents on the oral glucose tolerance in both normal and alloxan-induced diabetic rabbits. Jalal *et al.* (2007) has investigated the effect of the aqueous extract of shallots and garlic on fructose-induced insulin resistance rats. These results have showed that the shallots and garlic have a hypoglycemic affect. Importantly, aqueous shallot extract is a stronger hypoglycemic agent than the garlic extract. *Allium cepa* (onion) is also known to possess antioxidant and hypolipidaemic activity (Modak *et al*., 2007). Administration of a sulfur containing amino acid from onions, S-methyl cysteine sulphoxide, to alloxan-induced diabetic rats could significantly control the blood glucose and lipid levels in the serum, as well as normalizing the liver hexokinase, glucose-6-phosphatase and HMG Co A reductase (Roman-Ramos *et al*., 1995; Kumari *et al*., 1995).

1.6.1.3 Family Polygonaceae: *Polygonum odoratum*

Polygonum odoratum

Piper sarmentosum

Alpinia galangal

***Zingiber officinale* Rose**

Figure1.17 The plants of the spices and condiments used in this study

Polygonum odoratum is a culinary herb which is indigenous to Tropical south Asia. This plant belongs to neither the mint nor coriander families, but is instead a member of the same family as buckwheat and rhubarb (Hedges and Lister, 2007). This plant is believed to have a range of medicinal and other beneficial properties, such as antimicrobial properties, anti-inflammatory activities, antitumor-promoting activities and antioxidative properties (Shavandi *et al*., 2012). In 1997, Hunter *et al.* has reported that the constituents of the essential oil from Vietnamese coriander contained long-chain aldehydes, such as decanal (28%), dodecanal (44%), and decanol (11%). Sesquiterpenes (α-humulene, β-caryophyllene) accounted for about 15% of the essential oil. Numerous studies have shown good antioxidant activity of *P. odoratum* (Vimala *et al*., 2003; Huda-Faujan *et al.*, 2009). According to Nanasombat and Teckchuen (2009), *P. odoratum* contained flavonoids, such as rutin (3.77%), catechin (0.34%), quercetin (0.08%), isorhamnetin (0.01%) and kaempferol (0.01%), which all showed strong antioxidant activities.

1.6.1.4 Family Piperaceae: *Piper sarmentosum*

The leaves of *P. sarmentosum* have been used as food and in traditional medicine in Thailand. Previous studies have revealed the hypoglycemic effect of *P. sarmentosum* (Peungvicha *et al*., 1998; Noor *et al*., 1998). The ethanolic extract of *P. sarmentosum* leaves has been reported to decrease the blood sugar in alloxan diabetic rabbits (Pongmarutai, 1980). Moreover, this plant was able to reduce blood glucose level in moderately diabetic rats. Recently, Rukachaisirikul *et al.* (2004) has isolated and identified the chemical constituents in the fruit of *P. sarmentosum* containing of eight amides and two lignins.

1.6.1.5 Family Zingiberacea: *Alpinia galangal*and *Zingiber officinale* Rose

Alpinia galangal (galangal) and *Zingiber officinale* Rose (ginger) belong to the family Zingiberaceae and are commonly used as spices for culinary purposes and have been used in traditional medicines in Thailand in the relief of stomach aches, treating cold, invigorating the circulatory systems and reducing swelling. The main compounds of galangal extract are 1,8-cineole, β-bisaboline and β-selinene (Sookkongwaree *et al*., 2006). The rhizome of galangal also contains various flavonoids, such as kaempferol, kaempferide, galangin and alpinin (Charles *et al*., 1992). Several recent studies have suggested that these flavonoids might have a potent anti-cancer effect (Ciolino *etal*., 1999), while the rhizome of ginger contains over 20 phenolic compounds. The major constituents, including zingiberene, bisabolene, gingerols and shogaols, have been reported to possess diverse biological activities, such as antioxidant, anti-inflammatory, anticarcinogenic, antidiabetic, hypoglycemic, hypolipidimic and aldose reductase inhibitory activities (Al-Amin*et al.*, 2006). Ginger has also been reported to be effective in working against the development of diabetic cataract in rats (Saraswat *et al.*, 2010).

1.6.2 Fruits

Musa sapientum

Tamarind indica

Psidium guajava

Mangifera indica

Dimocarpus longan

Punica granatum

Figure 1.18 The plants used in this study

1.6.2.1 *Musa sapientum*

The isolated dietary fibre from bananas revealed significantly lower levels of fasting blood glucose levels and higher concentrations of liver glycogen in rats. Its soluble and insoluble components were believed to be involved in the hypo-cholesterolemic effect in rats. Freeze-dried banana pulp has also shown a marked cholesterol-lowering effect (Usha *et al*., 1989). The flowers of *Musa sapientum* are locally consumed as a fresh vegetable and used in Thai vegetable soups. It has been reported that administration of the fresh flower decoction could significantly decrease the hyperglycemic effect in rabbits. Moreover, the chloroform extract of the *M. sapientum* flower significantly reduced blood glucose levels and glycosylated hemoglobin levels in alloxanized rats. Antioxidant activity has also been reported (Pari and Maheswari, 2000).

1.6.2.2 *Tamarind indica*

The young shoots and leaves are locally consumed as a fresh vegetable and are also mixed in soups. A Thai traditional text book claims that the tamarind was used for wound healing, treatment of wounds, burns and wounds in diabetic patients (Farnsworth and Bunyapraphatsara, 1992). The ethanolic extract of *Tamarind indica* leaves showed a bactericidal effect against *Straphylococcus aureus*.

1.6.2.3 *Psidium guajava*

The ethanolic extract of *Psidium guajava* dried leaves showed the inhibition of hyperglycemia effects in alloxan-induced diabetic rats (Farnsworth and Bunyapraphatsara, 1992). Oh and colleagues (2005) have reported the antidiabetic effect of the guava leaf extract in the Type 2 diabetic mice model. Cheng and Yang (1983) reported on the potent antioxidant activity in guava leaf extracts and attributed this to its phenolic compounds. Numerous phenolic compounds were identified in guava leaf extracts as gallic acid, ferulic acid, chlorogenic acid, kaempferol, procatechuic acid, caffeic acid, quercetin and rutin (Wu *et al*., 2009).

1.6.2.4 *Mangifera indica*

Generally, the fruit of *Mangifera indica* is popularly consumed as a fruit. In Thailand, its young leaves are also consumed as a fresh vegetable. It is believed to decrease blood glucose level and cholesterol. The aqueous extract of leaves of *Mangifera* produced a reduction in blood glucose levels in glucose-induced hyperglycemia mice (Aderibigbo *et al*., 2001). *M. indica* has also been shown to powerful antioxidant activity *in vitro* (Martinez *et al.*, 2000). Chemical constituents in *M. indica*, especially phenolic compounds, flavonoids and triterpenoids, may be involved in the hypoglycemic effects (Ojewole, 2005). Muruganandan *et al*. (2005) has reported that mangiferin, which was isolated from *M. indica*, could decrease blood glucose level in STZ-induced diabetic rats and improve oral glucose tolerance in glucose loaded normal rats.

1.6.2.5 *Dimocarpus longan*

Longan belongs to the Sapindaceae family and is widely cultivated in Northern Thailand, Southern China. Longan seeds have traditionally been used in China for the treatment of acariasis, hernia wound hemorrhages, eczema and scrofula (Zheng, *et al*., 2012). Moreover, recent research studies have also been proven that longan seeds possesses free radical scavenging activity (Rangkadilok *et al*., 2007), growth inhibition of colorectal carcinoma cells (Chung *et al.*, 2010) and hypoglycemic effects (Huang *et al*., 2006). Soong and Barlow (2005) reported that the longan seed contained thirteen polyphenols, such as gallic acid, corilagin and ellagic acid, including chebulagic acid, ellagic acid 4-*O*-α-arabinofuranoside, isomalltinic acid and geraniin (Sudjaroen *et al*., 2012). Parts of the fruit and flower of the longan have also been shown to inhibit lipid peroxidation and free radical scavenging activities (Manohan *et al*., 2012).

1.6.2.6 *Punica granatum*

Consumption of concentrated pomegranate juice for 8 weeks in Type 2 diabetic patients could significantly reduce their total cholesterol, LDL-cholesterol, LDL-cholesterol/HDL-cholesterol and total cholesterol/HDL-cholesterol levels (Esmaillzadeh *et al.*, 2004). Oral administration of 50% ethanolic extract of *Punica* flowers resulted in blood glucose lowering effect in alloxanized diabetic rats (Jafri *et al*, 2000). This plant not only showed hypoglycemic and hypocholesterolemic activities, but also showed antioxidant activities. The pomegranate leaf was reported to contain high amounts of tannin and phenolic compounds and also to possess antioxidant activities (Cavalcanti *et al*., 2012; Gil *et al*., 2000). The antioxidant activities of pomegranate leaves might be attributed to the phenolic apigenin and luteolin glycosides.

1.6.3 Vegetables

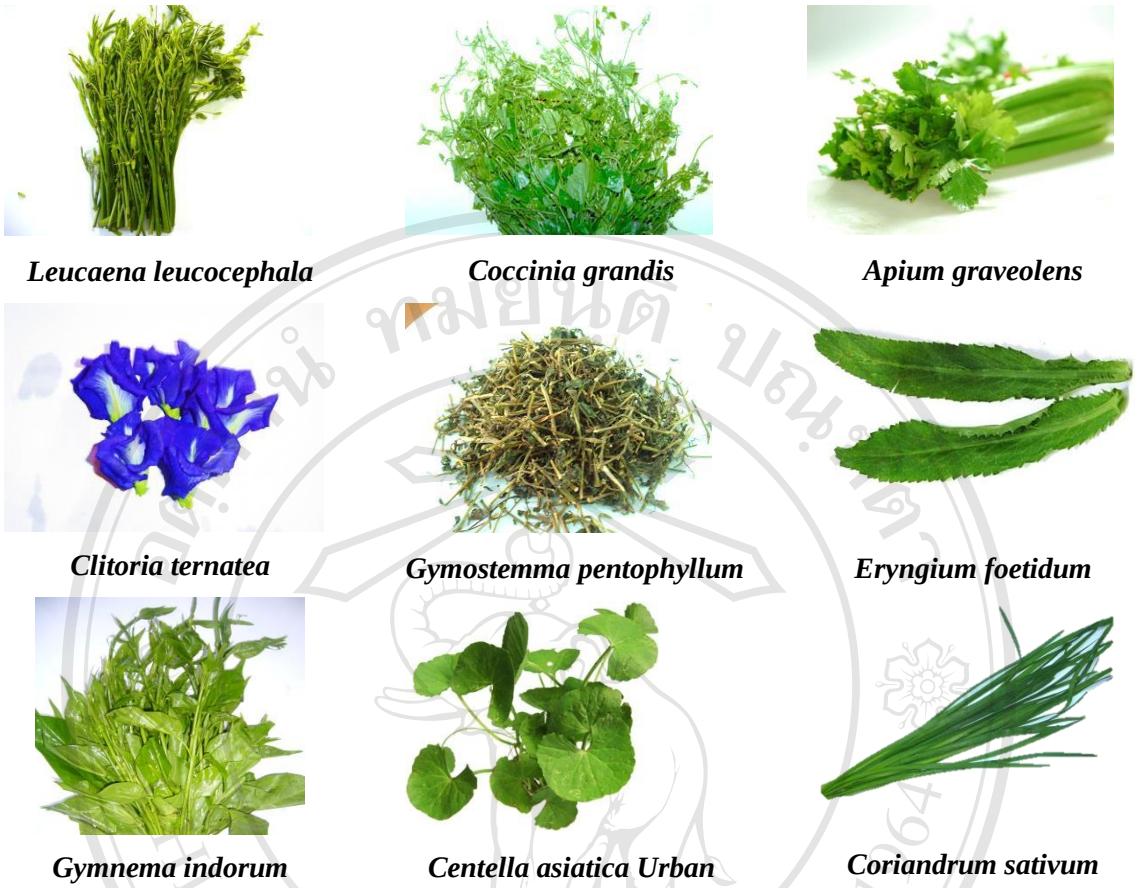


Figure 1.19 The plants (vegetables) used in this study

1.6.3.1 Family Leguminosae: *Leucaena leucocephala*and *Clitoria ternatea*

Clitoria ternatea Linn, belonging to the family Leguminosae, is a perennial twining herb found in India, China, the Philippines and Thailand. It has also been found to be useful in the treatment of severe fever, bronchitis and asthma (Asolkar *et al.*, 1992). The root has been used traditionally to induce abortion and its parts for curing abdominal swelling, sore throat, mucous disorders and fever (Devi *et al.*, 2003). Previous studies of the fresh flowers of *Clitoria ternatea* showed hypoglycaemic and hypolipidaemic effects in alloxan-induced diabetic rabbits (Rajathi and Daisy, 2000). The aqueous extract of *Clitoria ternatea* leaves and flower were confirmed for their antihyperglycaemic effects in alloxan-induced diabetic rats (Daisy and Rajathi, 2009).

Another plant in this family, *Leucaena leucocephala* (Lead tree) has been used as medicine and has been commonly consumed as a fresh vegetable in Thailand. In 2010, Syamsudin *et al.* reported that the active fraction of *L. leucocephala* seeds, which contained galactomanan, had antidiabetic activity in alloxan-induced rats.

1.6.3.2 Family Asclepiadaceae: *Gymnema indorum*

Gymnema indorum belonging to Asclepiadaceae is well known to have therapeutic effects in treating certain diseases, such as diabetes mellitus. In 1997, Shimizu *et al.* reported that the extract of *Gymnema indorum* leaves could suppress the glucose evoked-transmural potential and prevent a blood glucose increase in rats.

1.6.3.3 Family Cucurbitaceae: *Coccinia grandis*and *Gymostemma pentophyllum*

Coccinia grandis (ivy gourd) which belongs to the same family of *Gymostemma pentophyllum* (jiaogulan) as Cucurbitaceae, has been known to be a traditional herb and is popular in Asian countries. Kao *et al*., (2008) has revealed the comparative hypoglycemic activities of 30 hypoglycemic medicinal plants on alloxan-induced diabetic in rats. They found that the ethanolic extract of the ivy gourd leaves showed a significant blood glucose lowering effect in diabetic rats within 1week. The ethanolic of ivy gourd also showed antioxidant activity in streptozotocin diabetic rats. Whereas, *G. pentophyllum* (Jiaogulan) displayed several biological activities, such as inhibition of tumor cell growth (Yang *et al*., 2006), reduction of blood cholesterol and triacylglycerol levels (Xu *et al*., 2007) and protection of the liver (Megalli *et al*., 2005). The chemical composition of jiaogulancontains saponins and flavonoids were believed to be responsible for their biological activities (Kao *et al.,* 2008).

1.6.3.4 Family Umbeliferae: *Centella asiatica* Urban, *Apium graveolens, Eryngium foetidum* and *Coriandrum sativum*

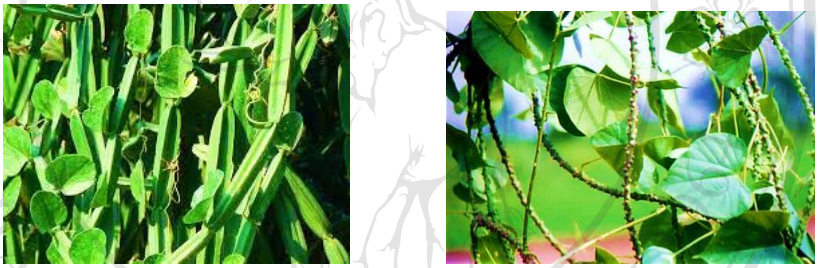
Penny wort (*Centella asiatica* Urban) is used in folk medicine to treat various health problems, such as wound healings (Kartnig, 1988), treating mental fatigue, anxiety and eczema (Goh *et al.*, 1995). Hamid and colleagues (2002) have

reported that the ethanolic extract of all parts of *C. asiatica* exhibited significantly strong antioxidative activities.

Coriander (*Coriandrum Sativum* L.) is a member of the Umbeliferae family. Coriander is an annual popular culinary medicinal plant with a distinctive pungent, fatty, and aldehydic aroma. Recently, Coriander oil has been reported to possess many medicinal properties, including antimicrobial properties, antioxidant, antidiabetic, anticancer and antimutagenic activities (Shavandi *et al*., 2012).

Wild coriander (*Eryngium foetidum*) is a culinary plant in Thailand and has also been used widely in herbal medicines. *E. foetidum* has been applied for its anti inflammatory activity, and to treat hypertension rheumatism, asthma, eye diseases, diabetes and digestive trouble.

1.6.4 Herbs



Cissus quadrangularis ***Andrographis paniculata* Wallex Nees**

Figure 1.20 The plants (herbs) used in this study.

1.6.4.1 Family Vitaceae: *Cissus quadrangularis*

C. quadrangularis has been widely used locally in the native system of medicines for various ailments in Thailand. The stem of *C. quadrangularis* has been used as a traditional medicine for such ailments as anthelmintic, dyspeptic, digestive disorders, as a tonic and as a analgestic in the eyes and ears. Several biological activities of *C. quadrangularis* were reported. Administration of the hydroalcoholic extract of *C. quadrangularis* showed a significant reduction in the blood glucose levels in alloxan-induced diabetes rats (Vijayakumari *et al*., 2012). Srivastava *et al.*, (2011) has also reported that flavonoids and terpenoids, which are the active constituents in the

stem of *C. quadrangularis* may be responsible for its hypoglycemic effect in alloxan-induced diabetes rats.

1.6.4.2 Family Acanthaceae: *Andrographis paniculata* Wallex Nees

Andrographis paniculata is a plant has been effectively used in a traditional Asian medicine in Thailand, China and India. Many disease conditions have been commonly treated with *A. paniculata* in traditional medical strategies, such as for antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic and adaptogenic effects. The water extract of *A. paniculata* markedly prevents diabetes in a glucose-induced hyperglycemia rabbits (Borhanuddin *et al.,* 1994). The extract also significantly lowered levels of thiobarbuturic acid-reactive substances in the liver and kidneys, while significantly increasing the activity of superoxide dismutase and catalase enzymes in rats (Zhang and Tan, 2000). The aerial part of *A. paniculata* contains a large number of diterpenes, lactones and flavonoids (Akbar, 2011).

1.7 Purpose and scope of this study

Currently, several Thai culinary plants containing large amounts of bioactive compounds, particularly phenolic and flavonoid compounds, have exhibited strong antioxidant properties. However, the phytochemical data of these plants with regard to their ability to alleviate or prevent diabetic and diabetic complications are scarce. Therefore, 26 culinary plants will be screened for total contents of phenolic and flavonoids compounds, as well as their antioxidant and antiglycation properties. The plants which show strong inhibitory activities will be selected for further investigation. The active plant will be separated, partially purified and identified by chromatography techniques. Some partially purified bioactive compounds, especially phenolic compounds from the active plant, will be investigated for their actions on antiglycation activities in different model proteins and anti-diabetic properties through the inhibition of α-glucosidase enzyme and compared with the selected standard phenolic compounds.