

Study on the antiglycation activities of young shoot leaves in nine edible plants used in Thai cuisine for health promotion

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ABSTRACT

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This study aimed to investigate the antiglycation activities of ethanol extract in nine young shoot leaves used in Thai cuisine. The highest total phenolic content was observed in *Cratoxylum formosum* (167.80 ± 1.61 mg GAE/g DW). The highest flavonoid content was found in *Azadirachta indica* (20.13 ± 0.51 mg QE/g DW), with the strongest antioxidant capacity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay exhibited in *Schinus terebinthifolius* (264.31 ± 25.45 mg AAE/g DW). The inhibition of advanced glycation end products (AGEs) of leaf extracts equivalent to 1 mg/mL based on dried herb weight was studied *in vitro* using the bovine serum albumin (BSA)-glucose model. The extracts showing more than 50% inhibition of total AGEs after incubation at 40 °C for seven days demonstrated significant antiglycation activity, consisting of *Cratoxylum formosum*, *Azadirachta indica*, *Sesbania grandiflora*, *Moringa oleifera*, *Schinus terebinthifolius*, and *Careya sphaerica*. *Cratoxylum formosum* leaf extract has the highest percentage of inhibition on certain compounds associated with oxidative stress and glycation. The following results were revealed: total AGEs ($82.84 \pm 0.93\%$), methylglyoxal-BSA ($97.21 \pm 0.29\%$), dityrosine ($98.59 \pm 0.26\%$), and pentosidine ($99.50 \pm 0.14\%$). The findings of this work might be useful for further study involving the examination of each plant in depth to identify complementary dietary benefits for health promotion.

Keywords: advanced glycation end products; *Cratoxylum formosum*; dityrosine; pentosidine; bovine serum albumin

1. INTRODUCTION

Glycation is a non-enzymatic chemical reaction that occurs spontaneously between reducing sugars such as glucose, fructose, ribose, and nucleophilic proteins. Glycation is a

natural process that occurs endogenously as part of normal metabolism. Reducing sugar (mainly glucose) reacts with a free amino group of proteins in lysine (Lys) and arginine (Arg) side chains, two basic amino acids that hold a positive charge at physiological pH. These reactions yield a

heterogeneous set of compounds known as advanced glycation end products (AGEs) (Uceda et al., 2024).

Glycation is a slow process; it starts with the reaction of a keto group of glucose or an α -hydroxyaldehyde and the Lys/Arg side chains. The formation of AGEs comprises multiple steps. Firstly, reducing sugars containing carbonyl groups react rapidly with free amino groups of proteins to form unstable products, known as Schiff bases. This adduct then undergoes rearrangement to form reversible and more stable Amadori products. These intermediate products undergo further irreversible oxidation or hydrolysis through a series of reactions known as the Hodge pathway to form varieties of AGEs. Other routes are the Namiki pathway and the Wolff pathway, constituting the formation of carbonyls after the autooxidation of monosaccharides (glucose, fructose, ribose, and glyceraldehyde). Namiki pathways convert Amadori products into highly reactive carbonyl species (RCS), such as glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosone (3-DG) (Khalid et al., 2022). The consequent RCS can further react with proteins (lysine/arginine) and diversify the AGEs. These reactions may take several days to weeks. This process is stimulated by the presence of high blood glucose levels in diabetes and occurs with various proteins such as collagen, cartilage, and red blood cells, including human serum albumin (HSA).

The accumulation of endogenous or exogenous AGEs is a cause of aging. There are no enzymes to remove glycated products from the human body; the excessively high levels of AGEs in tissues and the circulation can become pathogenic. AGEs can induce oxidative stress and proinflammatory effects and then activate various downstream processes that accelerate the progress of aging. High blood AGE levels have been linked to many diseases, including Alzheimer's, diabetes, cardiovascular diseases, and kidney failure. Patients with diabetes are at a higher risk of producing too many AGEs, which can then build up in the body and play an important role in the pathogenesis of diabetic complications like retinopathy and nephropathy (Singh et al., 2014; Khalid et al., 2022; Peng et al., 2024). Serum AGEs correspond to a unique common cancer risk factor and are used for the early detection of cancer (Turner, 2015). In addition, glycation can also occur on nucleic acids and aminophospholipids, as both possess free amino groups. Nucleic acids can be modified by reducing sugars and RCS to form DNA-AGEs, which impact the DNA structure and functionality. These highly oxidized compounds are also known as glycotoxins. The pathophysiological effects of AGEs are mainly due to oxidative stress and inflammatory processes. Reactive oxygen species (ROS) are formed endogenously during the mitochondrial respiratory cycle, as well as during cellular metabolism and nonenzymatically through transition-metal-ion-catalyzed redox reactions (Fenton reaction) (Reddy, 2023). Oxidative stress damages the structural modifications of proteins and nucleic acids. AGEs formed during the high-temperature processing of foods may also contribute to disease onset and many pathophysiologic symptoms. These endogenous and exogenous AGEs should be reduced by the daily intake of functional foods or other health promoters.

Currently, more than 20 different AGEs have been identified in human blood, tissues, and food (Twarda-Clapa et al., 2022). AGEs can be classified according to their origin, structure, and properties. They can be categorized into three groups: 1) fluorescent and cross-linked; 2)

nonfluorescent and cross-linked; and 3) nonfluorescent and noncross-linked proteins (Velichkova et al., 2021; Chen et al., 2022). Examples of well-characterized common AGEs are pentosidine, crossline, vesperlysine A, vesperlysine B, vesperlysine C, glyoxal-lysine dimer (GOLD), methylglyoxal-lysine dimer (MOLD), 3-deoxyglucosone-derived lysine dimer (DOLD), carboxymethyl-lysine (CML), carboxyethyl-lysine (CEL), pyrroline, and pyrroline imine (Perrone et al., 2020).

To inhibit the formation of AGEs, various methods have been proposed, such as reducing active dicarbonyl compounds, inhibiting ROS formation, protecting the protein structure, and inactivating AGEs. AGE inhibitors can be divided into roughly three classes: carbonyl-trapping agents, which diminish carbonyl stress and early glycation products; metal ion chelators, which suppress glycooxidation; and cross-link breakers, which reverse AGE cross-linking (Grzegorzczak-Karolak et al., 2016). Many synthetic inhibitors of AGE formation are not in clinical use due to unsatisfactory safety profiles.

Aminoguanidine (AMG), or the so-called pimagedine, is the first drug with clinical therapeutic potential in this regard. AMG is a nucleophilic hydrazine compound that inhibits the formation of AGEs by trapping the Amadori carbonyl group through nucleophilic addition reactions and stopping further rearrangement (Song et al., 2021). However, AMG has severe side effects that limit its clinical applications (Ahmed, 2005). AMG is usually used as a standard drug in the *in vitro* study of antiglycation. Aminoguanidine reacts with the physiological α -oxoaldehydes (glyoxal, methylglyoxal, and 3-deoxyglucosone) to form 3-amino-1,2,4-triazine derivatives that prevent glycation by those agents *in vitro* and *in vivo* (Thornalley et al., 2000).

Phenolic compounds present in fruits, vegetables, and herbs are known to provide strong antioxidant ability and are usually safe for human consumption. Phenolic compounds are considered to be among the most abundant classes of phytochemicals with health-promoting effects (Bolat et al., 2024). At present, there is increasing interest in finding natural antioxidants from plant sources to substitute synthetic chemicals. Antioxidants from plant foods are safe and varied in function. Many recent studies have explored the antiglycation properties of different plants to discover promising plant-derived compounds for use in medicine, food products, or cosmetics. Both whole plants and specific parts such as leaves, bark, bulbs, flowers, flower buds, and fruits have been investigated (Moe et al., 2022; Cushnie et al., 2024; Rodwattanagul et al., 2025; Neatpatiparn et al., 2025). Various plant extracts and purified components have been confirmed as high-potential AGE inhibitors (Sadowska-Bartos & Bartosz, 2015). The inhibition of AGEs may be another mode of diabetes treatment or slow down the effects of aging. In Thailand, numerous edible plants exist in certain types of trees and are famous only in specific areas of the country. The edible parts might be available in different periods of the year and are mostly used as vegetable side dishes. In this present study, nine plants were selected because the leaves of these trees have been used as traditional Thai food for a considerable time. Perennial trees are more sustainable than annual vegetable plants. Some are better known as medicinal herbs than edible vegetables. To highlight the role of these plants as edible leaves, with a long history of being safe for consumption, the antiglycation properties of leaves from selected perennial trees were evaluated via *in vitro* studies

using the BSA-glucose model to search for potential leaves from nature to resolve the problem of glycation.

The purpose of this study was to determine the antiglycation activity, total phenolic content, total flavonoid content, and total tannin content of young shoot leaves from eight Thai indigenous plants and one popular non-native plant, *Schinus terebinthifolius* (Brazilian pepper tree), as presented in Table 1. All selected plants are perennial trees except *Hibiscus sabdariffa* L., which is a hardy herbaceous shrub with surprisingly edible young shoot leaves, and is usually cultivated for its calyces.

2. MATERIALS AND METHODS

2.1 Materials

Bovine serum albumin (BSA) was purchased from Himedia Laboratory Pvt., Ltd., India, and aminoguanidine hydrochloride (AMG) from Glentham Life Sciences Ltd., UK. Sodium azide was purchased from Fujifilm Wako Pure Chemical Corporation, Japan. Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and quercetin were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA). All other chemicals were of analytical reagent (AR) grade.

2.2 Plant preparation and extraction

Fresh young shoot leaves (Figure 1) were collected from the garden at Huachiew Chalermprakiet University, Samut Prakan, or purchased from a local fresh market near the university during September to December 2024. The scientific names of the plant samples were then authenticated as shown in Table 1. The specimens were deposited at the Pharmacognosy and Pharmaceutical Chemistry Laboratory, Huachiew Chalermprakiet University.

The edible young shoot of each plant was washed, dried at 60 °C for 4 h in a hot air oven, and then pulverized. The size was controlled by passing the pulverized plant through a standard sieve No. 16. One gram of each plant was weighed and soaked with 10 mL of 75% ethyl alcohol at room temperature for 24 h. The samples were sonicated for 20 min at 35–37 °C, and the supernatant was then collected after centrifugation at 3500 rpm for 10 min. The residual leaf residues (marc) were re-extracted four times with a fresh 5.0 mL of 75% ethyl alcohol. All supernatants were pooled and filtered using a syringe filter (0.45 µm) and transferred into a tight container for storing at 4–8 °C until use. The resulting liquid extracts corresponded to 1 µg of dry powder per 30 µL. Each plant was extracted in triplicate.

Table 1. Scientific name, common names, and botanical families of selected plants

Scientific name	Common name	Family
<i>Azadirachta indica</i> A. Juss.	Neem	Meliaceae
<i>Careya sphaerica</i> Roxb.	Wild guava	Lecythidaceae
<i>Cratoxylum formosum</i> (Jacq.) Benth. & Hook. f. ex Dyer	Mempat	Hypericaceae
<i>Hibiscus sabdariffa</i> L.	Roselle	Malvaceae
<i>Moringa oleifera</i> Lam.	Horse radish tree	Moringaceae
<i>Morus alba</i> L.	White mulberry	Moraceae
<i>Schinus terebinthifolius</i>	Brazilian pepper tree	Anacardiaceae
<i>Sesbania grandiflora</i> (L.) Poir.	Hummingbird tree	Fabaceae
<i>Syzygium antisepticum</i> (Blume) Merr. & L. M. Perry	Shore eugenia	Myrtaceae



Figure 1. The young shoot leaves of nine edible plants used in Thai cuisine as vegetables

2.3 Determination of total phenolic content

The total phenolic content of each plant extract was determined using the Folin-Ciocalteu assay described by Chewchinda et al. (2019) with modifications. Briefly, 10 μ L of the extract (or 3 μ L for *C. sphaerica*, *C. formosum*, *S. terebinthifolius*, and *S. antisepticum*) with sufficient deionized water to make 50 μ L was mixed with 1000 μ L of 10% Folin-Ciocalteu's solution and incubated for 5 min. Then 950 μ L of 6.5% w/v sodium carbonate solution was added and incubated for 45 min in the dark at room temperature. The absorbance was measured (UV 1800, Shimadzu, Japan) using a spectrophotometer at 760 nm against a blank containing all reagents except the plant extracts. A gallic acid stock solution (500 μ g/mL) was used to establish the standard curve. Aliquots of the stock solution (5, 10, 20, 30, 40, and 50 μ L) were transferred into six 2-mL microcentrifuge tubes with the addition of sufficient deionized water to obtain a final volume of 50 μ L. Then, 1000 μ L of 10% Folin-Ciocalteu's solution and 950 μ L of 6.5% w/v sodium carbonate solution were added and incubated in the same manner as the test samples. The total phenolic content of the extracts was expressed as milligrams of gallic acid equivalent per gram of dry powder (mg GAE/g DW).

2.4 Determination of total flavonoid content

The total flavonoid content was measured by the aluminum chloride colorimetric method described by Shraim et al. (2021) with modification. In a 2-mL microcentrifuge tube, 30 μ L of each extract and 70 μ L of deionized water were mixed with 1000 μ L of 10% aluminum chloride. After 5 min, 900 μ L of 1 M potassium acetate was added and incubated for 30 min in the dark at room temperature. The absorbance was measured using a spectrophotometer at 425 nm against a blank containing all reagents except the plant extract. A quercetin stock solution (500 μ g/mL) was used to establish the standard curve by aliquoting it into 10, 20, 30, 40, 50, and 60 μ L, with the same process performed as for the test samples. The flavonoid content of the extracts was expressed as milligrams of quercetin equivalent per gram of dry powder (mg QE/g DW).

2.5 Determination of total tannin content

The total tannin content was estimated using the methylcellulose precipitable tannin assay method described by Li et al. (2023) with modification. Briefly, 1000 μ L of 0.4% methylcellulose solution was added to 25 μ L of the plant extract diluted with 475 μ L of deionized water. After gentle shaking and 10 min of incubation at room temperature, 1000 μ L of ammonium sulfate solution (39% w/v) was added, followed by 2.5 mL of deionized water. The supernatant was withdrawn and centrifuged at 12000 rpm for 10 min. In the control tube, 1000 μ L of deionized water was used instead of the methyl cellulose solution. The standard curve of aqueous (-)-epicatechin solution was constructed from the concentrations of 1, 5, 10, 25, 50, and 100 μ g/mL. The absorbance was measured using a spectrophotometer at 280 nm against a blank containing all reagents except the plant extract. The total tannin in the test samples was calculated according to the formula $C_{TT} = (C_c - C_T)$, where C_{TT} represents the total tannin content of the test sample, C_c is the control tube, and C_T is the phenolic content after precipitating the total tannin. The total tannin content in the extracts was expressed as milligrams of epicatechin equivalent per gram of dry powder (mg EE/g DW).

2.6 Determination of DPPH free radical scavenging

The DPPH free radical scavenging assay was carried out according to the method described by Baliyan et al. (2022) with modification. Ascorbic acid was used as a standard antioxidant. A series of standard solutions was prepared from a stock solution (250 μ g/mL) by aliquoting 5, 10, 20, 30, 40, and 50 μ L and placing them in a 2-mL microcentrifuge tube. Then, 1.5 mL of DPPH solution (0.004% w/v in 80% ethanol) was added, along with sufficient deionized water to make a final volume of 2.0 mL. The mixture was allowed to stand in the dark at room temperature for 30 min. The absorbance at 520 nm was measured against a control solution containing all reagents except the extract, using a spectrophotometer.

In the test sample, 10 μ L of the extract was aliquoted, except for *C. sphaerica*, *C. formosum*, *S. terebinthifolius*, and *S. antisepticum*, which were diluted 10 times before being aliquoted for 10 μ L and subjected to the same procedure as the standard solutions. The percentage inhibition of the DPPH radical by both the ascorbic acid standards and plant extracts was calculated using the following formula:

$$\% \text{ inhibition} = [(A_c - A_s)/A_c] \times 100$$

where A_c represents the absorbance of the control solution and A_s the absorbance of the test solution. The DPPH radical scavenging capacity of the extracts was calculated as milligrams of ascorbic acid equivalent per gram of dry weight (mg AAE/g DW) using the calibration curve constructed by plotting the percentage inhibition versus the concentration of ascorbic acid.

2.7 Antiglycation study

2.7.1 Adjustment of the glycation system

To identify an assortment of procedures from the literature and research works, the formation of AGEs was assessed using the BSA-glucose model. Briefly, 10 mg/mL of BSA was dissolved in pH 7.4 phosphate-buffered saline (PBS) containing 0.02% sodium azide as a preservative and incubated with or without a glucose solution ranging from 50 to 250 μ M. The total glycation volume consisted of 4.5 mL in a 5-mL polypropylene tube. Incubation temperatures were trialed at 37 °C and 40 °C, and the sample was withdrawn for measuring on days 0, 1, 4, 7, 21, and 28. To investigate the formation of AGEs, vesperlysine-like AGEs and pentosidine-like AGEs were examined at ex370/em440 nm and ex325/em440 nm, respectively. The fluorescence intensity of AGEs was measured using a disposable polymethyl methacrylate cuvette and a spectrofluorometer (Thermo Scientific Lumina, Thermo Fisher Scientific Inc., USA). The analysis was conducted using a photomultiplier tube voltage of 600 V, the excitation and emission slit widths were 5 nm, and the integration time was 20 milliseconds.

2.7.2 Antiglycation study

Stock solutions of BSA (20 mg/mL) and glucose (750 mM) were separately prepared in pH 7.4 PBS containing 0.02% sodium azide. Then, 45 μ L of each plant extract was incubated with 750 μ L of BSA and 500 μ L of glucose solution in a 2-mL microcentrifuge tube. PBS was used to adjust the final volume to 1.5 mL. A control tube contained 750 μ L of BSA, 500 μ L of glucose solution, and 250 μ L of PBS. Aminoguanidine HCl, quercetin, and gallic acid at the final concentration of 1 mM were used as positive controls

and benchmarks of natural compounds. All tubes were incubated for 7–14 days at 40 °C in a temperature-controlled cabinet. Four parameters associated with glycooxidation were measured: total AGEs, fluorescence intensity at ex370/em440 nm (Kaewnarin et al., 2014), and methylglyoxal-BSA (MGO-BSA) at ex340/em420 nm (Koike et al., 2023). Dityrosine was measured at ex330/em415 nm and pentosidine at ex335/em385 nm. Antiglycation activity was determined from the decrease in fluorescence intensity. The percentage of AGE inhibition was calculated using the following equation:

$$\text{Inhibition (\%)} = [(F_{\text{gBSA}} - F_{\text{eBSA}}) / F_{\text{gBSA}}] \times 100\%$$

where F_{gBSA} represents the fluorescence intensity of the glycated BSA (control) and F_{eBSA} the glycated BSA incubated with plant extract.

2.8 The effect of herbal extracts on protein conformation and quenching

The structural changes of BSA induced by plant extracts were characterized using fluorospectroscopic techniques. Protein conformation was detected using tryptophan fluorescence quenching. The fluorescence emission profiles of all reaction tubes after incubation at 40 °C for seven days were diluted 10 times with PBS; thus, they contained 1 mg/mL of BSA, and the leaf extract corresponded to 0.1 mg/mL. The excitation wavelength was 280 nm, and the fluorescence spectrum was scanned at 250–500 nm.

2.9 Statistical analysis

All experiments were carried out in triplicate, and the data were expressed as mean values $\pm$ SD. The equations of the calibration curves were calculated by linear regression analysis. The significance of differences between test samples and the control was tested by the ANOVA post hoc test (Bonferroni correction) using Stata (18.5 BE-basic edition, Statacorp LLC, USA) software. The correlation between two variants was analyzed by the Pearson test. The significance value was set at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Plant extraction

Extraction was performed using 75% ethanol as a safe and effective solvent for extracting phenolic compounds and flavonoids from plant materials. To reduce the step of evaporation and redissolution of the extract, the final concentration was projected to be 1 mg/30 μ L based on the dry weight of the plant powder. This method is appropriate for rapid screening and comparative study. Extraction using ultrasonic-assisted extraction (UAE) is recognized as an environmentally friendly and highly efficient technology. This method minimizes the amount of organic solvent, thereby reducing the impact on the environment. In addition, the UAE has been found to significantly enhance the extraction of bioactive components, making it an interesting method in the industry and related fields (Shen et al., 2023).

3.2 Glycation protocol

In vitro glycation reactions have been reported using many types of proteins and reducing sugars. To seek a suitable protocol for this research, a kinetic study was conducted to

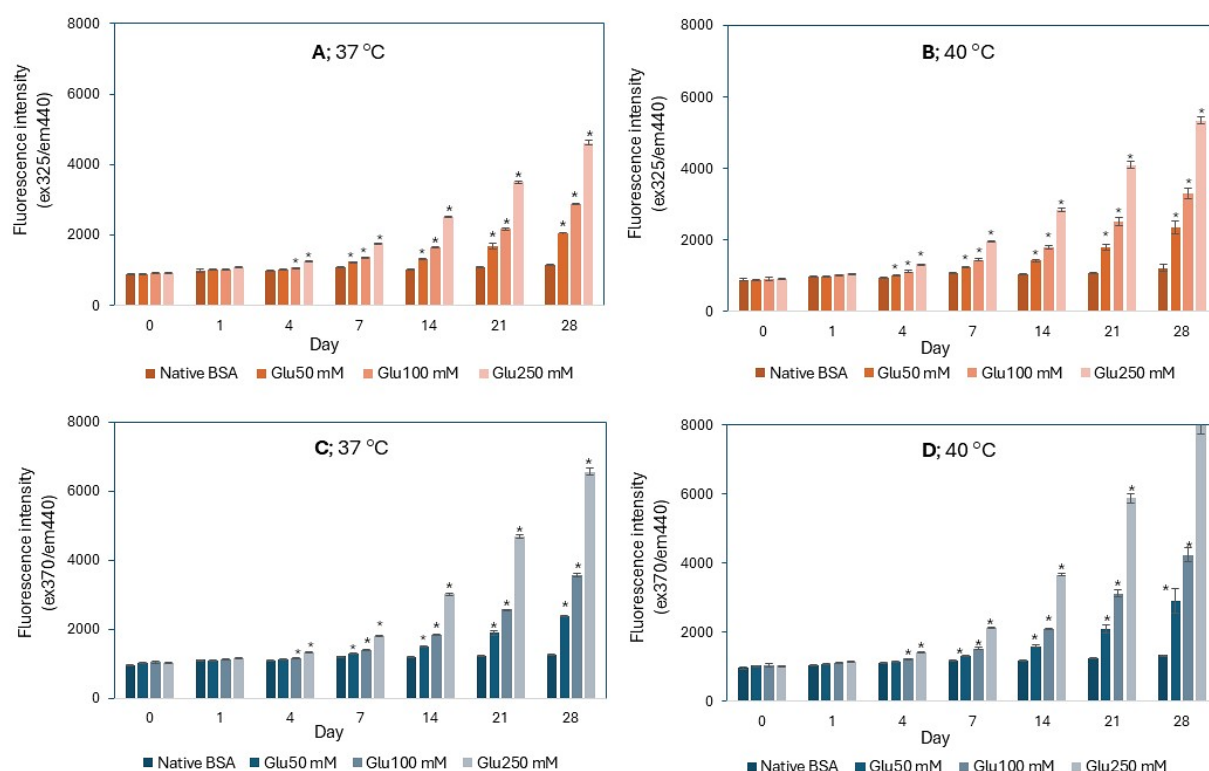
track the formation of AGEs using the BSA-glucose model. BSA is the most frequently used due to its high sequence homology to HSA.

Sadowska-Bartosz and Bartosz (2015) studied the kinetics of the glycooxidation of BSA using three sugars: glucose, fructose, and ribose. They found that different sugars resulted in highly different rates and extents of AGEs' formation. Ribose showed the highest effect on AGEs' formation, followed by fructose and glucose. Thus, caution should be taken when comparing the results between one sugar and another. In fact, glucose is the main glycation sugar and is present in the human body at the highest concentration. The normal fasting blood glucose level is between 3.9 and 5.6 mM. While a level from 5.6 to 6.9 mM is considered prediabetes, a level of 7.0 mM or higher on two separate tests indicates diabetes (WHO, 1999). Normal serum fructose concentrations in healthy persons are only $8.1 \pm 1.0 \mu\text{M}$, while they are $12.0 \pm 3.8 \mu\text{M}$ in diabetes patients. Hence, D-glucose was chosen for this study. According to the literature review, the glycation reactions differ from actual values and vary in both the concentration of substances used and the incubation temperature. To understand and conduct the glycation reaction to fit with the experiment and facilities in this study, BSA (10 mg/mL) was incubated at 37 °C and 40 °C in the presence of glucose (50, 100, and 250 mM) and monitored for 28 days.

Figure 2 shows the fluorescence intensity monitored at wavelengths of ex325/em440 nm and ex370/em440 nm versus time. Under identical conditions, native BSA contained the intrinsic fluorescence intensity of AGEs. These intrinsic AGEs did not increase significantly after incubation for 28 days ($p > 0.05$). An increase in the fluorescence intensity of glycated BSA when compared to its native BSA was observed. Significantly higher formation of AGEs by glycated BSA compared to native BSA was first found at day four in the reaction tube containing at least 50 mM of glucose, at an incubation temperature of 40 °C measured at ex325/em440 nm ($p < 0.01$). After day four with a glucose concentration of 50 mM in all conditions, the intensities of AGEs were significantly higher than native BSA ($p < 0.01$). The kinetics of fluorescence intensity increased along with glucose concentration, incubation time, and temperature. Measured wavelengths of ex370/em440 nm were more often found in the literature compared to ex325/em440 nm. Wavelengths of ex370/em440 nm have been suggested for measuring vesperlysine-like AGEs, while wavelengths of ex325/em440 nm were used to measure pentosidine-like AGEs (Séro et al., 2013).

Vesperlysines (types A, B, and C) are cross-linked products generated from two lysine side chains in proteins and the oxidative degradation of glucose (Nakamura et al., 1997); nonetheless, both are glycooxidation end products. In this present study, wavelengths of ex370/em440 nm were used to estimate the formation of AGEs due to the increasing rate and higher fluorescence intensity compared to those measured from ex325/em440 nm. The excitation at 370 nm might be subject to less interference from the absorption of plant phytochemicals. An incubation temperature of 40 °C and 250 mM of glucose accelerated the AGEs' formation rate, suggesting the suitability of this method for instantaneously screening many extracts where binding competition between sugar molecules and the bioactive compounds might occur.





*Values significantly higher than native BSA ($p < 0.01$)

Figure 2. The time course of the glycation of BSA incubated at 37 °C (A, C) and 40 °C (B, D) for 0–28 days to investigate the effect of glucose concentrations (0–250 mM) on the formation of AGEs of BSA.

Note: Fluorescence intensities were measured at two wavelength pairs: ex325/em440 nm (A, B) and ex370/em440 nm (C, D). Data is the mean of three independent experiments. Error bars represent standard deviation.

3.3 Total phenolic, total flavonoid, and total tannin content

Phytochemicals are secondary metabolites produced by plants known for their medicinal benefits in both traditional and modern medicines. Phenolic compounds are a large group of phytochemicals with hundreds of substances, including flavonoids and tannins. The existence of numerous hydroxyl groups in phenolic compounds is responsible for their antioxidant properties. Phenolic compounds have been reported to bind to microbial cell membranes, disrupting the membrane structure and inhibiting the synthesis of nucleic acids and organelles within cells (Takó et al., 2020); thus, this group of plant metabolites possesses various bioactivities, including antiglycation. The mechanism underlying these activities involves the binding of phenolic compounds to serum albumin through hydrogen bonding or hydrophobic interactions, which may interfere with the glycation process and help preserve the protein structure (Oso et al., 2023). Various studies confirm that phenolic compounds bind to BSA through hydrophobic and hydrogen bonding (Tang et al., 2020; Rashedinia et al., 2023; Shahidi & Dissanayaka, 2023). Hydrogen bonds often form between the hydroxyl groups of polyphenols and carbonyl groups in proteins. Hydrophobic interactions mainly occur between the benzene rings of polyphenols and the nonpolar amino acids in proteins (Xue et al., 2024).

The total phenolic content was determined by the Folin-Ciocalteu method using gallic acid as a standard. The regression equation of the standard curve was Abs =

$(0.0947 \times \text{gallic acid concentration}) + 0.0181$, with $R^2 = 0.9999$. The total phenolic content of ethanolic extracts was expressed as milligrams of gallic acid equivalents per gram of sample in dry weight (mg GAE/g DW), as shown in Table 2. A significantly higher total phenolic content in mg GAE/g DW was found in *C. formosum* than in other samples (167.80 ± 1.61 , $p < 0.01$), followed by *S. terebinthifolius* (149.03 ± 13.92), *C. sphaerica* (124.80 ± 2.61), and *S. antisepticum* (97.20 ± 2.95), respectively. The lowest total phenolic content was recorded in *M. alba* and *S. grandiflora*, with values of 18.63 ± 0.72 and 19.03 ± 1.01 mg GAE/g DW, respectively.

The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve: Abs = $(0.0675 \times \text{quercetin concentration}) - 0.0044$ ($R^2 = 0.9996$) and expressed as milligram quercetin equivalents per gram of sample in dry weight (mg QE/g DW). The total flavonoid content results of each extract are provided in Table 2. Among the ten samples, *A. indica* leaf extract had the highest TFC (20.13 ± 0.51 mg QE/g DW), followed by *S. terebinthifolius* (15.70 ± 0.30), *C. formosum* (14.03 ± 1.22), and *S. antisepticum* (11.87 ± 0.51), respectively, with statistically significant differences between them ($p < 0.01$).

Tannins are important secondary metabolites that exist in all parts of plants. Tannins have been used throughout history for their pharmacological properties as part of plants and herbs in traditional medicine. Nowadays, tannins are well known for their excellent antioxidant and antibacterial activities (de Araújo et al., 2021). The total tannin content of the nine leaf extracts and neem flower was calculated from the regression equation of the calibration

curve: $\text{Abs} = (0.015 \times \text{epicatechin concentration}) + 0.0023$ ($R^2 = 1.000$) and expressed as milligram epicatechin equivalent per gram of sample in dry weight (mg EE/g DW).

The results for the total tannin content of each extract are presented in Table 2. *S. terebinthifolius* leaf extract contained the highest total tannin content, followed by the leaf extracts of *C. sphaerica*, *C. formosum*, and *S.*

antisepticum, with values of 307.80 ± 14.82 , 305.07 ± 22.31 , 210.73 ± 7.94 , and 174.47 ± 12.40 mg EE/g DW, respectively. The differences between these samples and the others were statistically significant ($p < 0.01$). These four plants possessed high astringency, a characteristic of high tannin content. Other samples were found to have lesser amounts, ranging from 25.75 ± 1.10 to 60.75 ± 3.57 mg EE/g DW.

Table 2. Total phenolic, total flavonoid, and total tannin contents and DPPH· scavenging activity of leaf extracts

Scientific name	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TT (mg EE/g DW)	DPPH· scavenging activity (mg AAE/g DW)
<i>A. indica</i> (flower)	$31.37 \pm 0.90^{a,b}$	$5.47 \pm 0.31^{a,b}$	31.61 ± 2.13^a	$30.70 \pm 0.37^{a,b}$
<i>A. indica</i> (leaf)	$50.27 \pm 2.64^*$	20.13 ± 0.51	$60.75 \pm 3.57^{a,*,\#}$	$53.52 \pm 0.39^{a,*}$
<i>C. sphaerica</i>	124.80 ± 2.61	$7.97 \pm 0.59^{a,c,*}$	305.07 ± 22.30^b	203.86 ± 21.64^c
<i>C. formosum</i>	167.80 ± 1.61	$14.03 \pm 1.22^{d,e}$	210.73 ± 7.94	124.48 ± 15.57
<i>H. sabdariffa</i>	$26.33 \pm 1.00^{a,c}$	$8.10 \pm 0.35^{a,c,\#}$	$25.97 \pm 3.59^{a,*}$	$18.10 \pm 0.77^{a,b}$
<i>M. oleifera</i>	$35.47 \pm 1.78^{a,*,\#,\$}$	$10.83 \pm 0.50^{f,*,\#}$	37.88 ± 2.15^a	$21.78 \pm 1.25^{a,b}$
<i>M. alba</i>	$18.63 \pm 0.72^{b,c,\#}$	$7.57 \pm 2.06^{a,c}$	$25.75 \pm 1.10^{a,\#}$	$16.09 \pm 1.08^{b,*}$
<i>S. terebinthifolius</i>	149.03 ± 13.92	15.70 ± 0.30^d	307.80 ± 14.82^b	264.31 ± 25.45
<i>S. grandiflora</i>	$19.03 \pm 1.01^{b,c,\$}$	4.07 ± 0.31^b	31.49 ± 2.36^a	3.18 ± 0.43^b
<i>S. antisepticum</i>	97.20 ± 2.95	$11.87 \pm 0.51^{e,f}$	174.47 ± 12.40	180.66 ± 1.11^c

Data represent mean $\pm$ SD of three independent experiments

Note: Values sharing the same letter are not significantly different. Values showing *, #, or \$ indicate significant differences at $p < 0.05$, while those without indicate highly significant differences ($p < 0.01$).

3.4 Antioxidant capacity

The antioxidant capacity of the extracts was investigated by the DPPH radical scavenging assay. This method is considered to be an ideal, easy, reliable, and reproducible colorimetric method for evaluating the antioxidant properties of antioxidant compounds or plant extracts (Gulcin & Alwasel, 2023). The DPPH radical has a deep violet color in solution, becoming colorless or pale yellow when mixed with a solution of a substance capable of donating a hydrogen atom, resulting in the reduced form of DPPH-H. The standard antioxidant was ascorbic acid solution (0.625 – 6.25 $\mu\text{g/mL}$). The calibration curve of DPPH· scavenging was percentage DPPH inhibition = $(12.773 \times \text{ascorbic acid concentration}) - 1.9306$, with $R^2 = 0.9981$. The concentration of ascorbic acid inhibiting 50% of the DPPH radical from this calibration curve was 4.06 $\mu\text{g/mL}$. The antioxidant capacity of the extracts was calculated and expressed as the milligram ascorbic acid equivalent per gram of dry weight (mg AAE/g DW) values, as shown in Table 2. The higher AAE value indicates a higher free radical scavenging capacity. The highest AAE value in mg/g DW was obtained from the extract of *S. terebinthifolius* (264.31 ± 25.45 , $p < 0.01$), followed by those of *C. sphaerica* (203.86 ± 21.64), *S. antisepticum* (180.66 ± 1.11), and *C. formosum* (124.48 ± 15.57), respectively. The lowest value was found in the extracts of *S. grandiflora* (3.18 ± 0.43).

3.5 Antiglycation activity

The antiglycation activity of the extracts was investigated using the BSA-glucose model from the prior study. The final concentration of the extracts was 1 $\mu\text{g/mL}$ based on the dry weight of plant powder. Aminoguanidine HCl (AMG) was used as a positive control; quercetin and gallic acid were also used as references for their antiglycation

activity. Figure 3 demonstrates the results expressed as the fluorescence intensity of AGEs measured at days 7 and 14. Native BSA showed its intrinsic early stage and AGEs naturally existing in the raw material, but the fluorescence intensity did not significantly increase with incubation time. In contrast, the fluorescence intensity of glycated BSA (gBSA) showed about a two-to four fold increase in incubation time.

Protein glycation is a distinct chemical reaction characterized by the covalent attachment of reducing sugars to amino acid residues, with lysine being the primary target. BSA is a single polypeptide consisting of 59 lysine residues, 30–35 of which have primary amines that can react with crosslinkers to couple peptides. The glycation of BSA also occurs in arginine and cystine residues (Rahman & Khalil, 2022). When the gBSA is incubated with the plant extract and positive controls, the lowering in fluorescence intensity of AGEs suppresses or traps the glycation. AMG is the first drug with clinical therapeutics to inhibit the formation of AGEs by trapping the carbonyl group of Amadori products through the addition of nucleophilic reactions and inhibiting their further rearrangement. AMG can also bind to active α -dicarbonyl intermediates (e.g., MGO), forming triazines, thereby blocking the transformation of Amadori products to AGEs (Song et al. 2021). In clinical settings, the use of AMG has been discontinued due to a high dose being required, resulting in side effects. In this study, AMG (1 mM) showed a moderate percentage inhibition of total AGEs ($17.47 \pm 1.41\%$) on day 7 and increased on day 14. Quercetin (1 mM) showed the highest inhibition for all measurements and was more effective than AMG and gallic acid. According to a previous study, the half-maximal inhibitory concentration (IC_{50}) of quercetin was 50 times higher than that of AMG for the inhibition of vesperlysine-

like AGEs (ex370/em440) after incubation for 24 h in the BSA-ribose model. Gallic acid (1 mM) was more effective

for pentosidine inhibition than other glycated products (Séro et al., 2013).

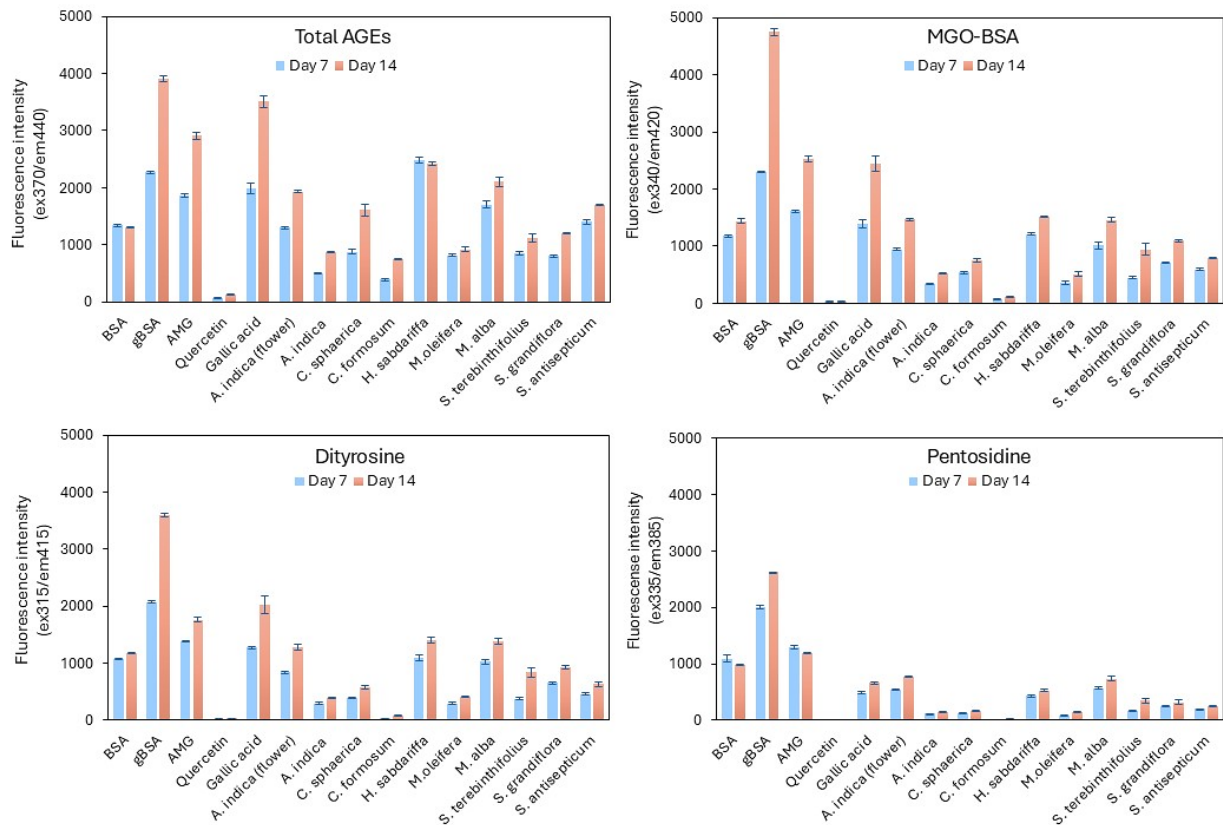


Figure 3. Fluorescence intensity (AU) of *in vitro* glycated BSA with and without the nine young shoot leaf extracts and one flower extract (1 µg/mL), quercetin (1 mM), and gallic acid (1 mM) and AMG (1 mM)

Note: Fluorescence intensity refers to the mean of three independent experiments. Error bars represent the standard deviation.

Table 3. Antiglycation activity (expressed as % inhibition) of nine leaf extracts and one flower extract and some natural substances on the AGE formation determined by the BSA-glucose assay after incubating at 40 °C for 7 days

	Total AGEs (ex370/em440)	MGO-BSA (ex340/em420)	Dityrosine (ex330/em415)	Pentosidine (ex335/em385)
Glycate BSA	0.00	0.00	0.00	0.00
AMG	17.47 ± 1.41	30.02 ± 0.94	33.30 ± 0.76	35.42 ± 1.58
Quercetin	96.84 ± 0.46	98.69 ± 0.09	99.21 ± 0.33	99.74 ± 0.19
Gallic acid	12.16 ± 3.84	39.71 ± 2.76	38.80 ± 1.37	75.93 ± 1.11
<i>A. indica</i> (flower)	42.82 ± 0.69	59.05 ± 1.10	59.39 ± 0.96	72.44 ± 0.05
<i>A. indica</i> (leaf)	78.07 ± 0.72	85.40 ± 0.47 ^a	85.79 ± 0.72 ^a	94.29 ± 0.32 ^a
<i>C. sphaerica</i>	61.42 ± 1.77 ^a	76.65 ± 1.10 ^{b,*}	81.36 ± 0.12 ^{b,*}	93.86 ± 0.92 ^{a,*}
<i>C. formosum</i>	82.84 ± 0.93	97.21 ± 0.29	98.59 ± 0.26	99.50 ± 0.14
<i>H. sabdariffa</i>	-9.25 ± 2.40	47.52 ± 0.97	47.68 ± 2.52	78.46 ± 0.73
<i>M. oleifera</i>	63.94 ± 1.19 ^a	84.27 ± 1.07 ^{a,#}	85.95 ± 0.95 ^{a,*,#}	95.45 ± 0.40 ^a
<i>M. alba</i>	24.75 ± 2.70	56.05 ± 2.49	50.55 ± 2.19	71.50 ± 0.71
<i>S. terebinthifolius</i>	62.18 ± 1.37 ^a	80.40 ± 1.02 ^{*,#}	81.78 ± 0.88 ^{b,c,#}	91.57 ± 0.64 ^{b,*}
<i>S. grandiflora</i>	64.73 ± 1.10 ^a	69.15 ± 0.21	68.71 ± 1.24	87.64 ± 0.37 [#]
<i>S. antisepticum</i>	38.16 ± 1.71	74.29 ± 0.63 ^b	78.00 ± 0.82 ^{b,c}	90.06 ± 0.23 ^{b,#}

Each value represents the mean ± SD (n = 3)

Note: Values sharing the same letter are not significantly different. Values with * or # indicate significant differences ($p < 0.05$), while those without indicate highly significant differences ($p < 0.01$) compared to each other and to aminoguanidine hydrochloride. Quercetin, gallic acid, and *A. indica* flower extract were excluded from this test.

Table 3 demonstrates the percentage inhibition of four glycosylated BSA parameters. The total AGEs or vespersylsine-like AGEs, are formed in the Maillard reaction between N α -acetyl-L-lysine and glyceraldehyde, an intermediate compound in carbohydrate metabolism. Vespersylsine-like AGEs were measured with excitation at 370 nm and emission at 440 nm (Séro et al., 2013). As shown in Table 3, inhibition greater than 50% was found from *C. formosum* ($82.84 \pm 0.93\%$), followed by *A. indica* (leaf) ($78.07 \pm 0.72\%$), *S. grandiflora* ($64.73 \pm 1.10\%$), *M. oleifera* ($63.94 \pm 1.19\%$), *S. terebinthifolius* ($62.18 \pm 1.37\%$), and *C. sphaerica* ($61.42 \pm 1.77\%$), respectively.

MGO is the major precursor for the formation of AGEs in the human body. MGO is a highly reactive α -dicarbonyl compound in the Maillard reaction, leading to the formation of stable, irreversible protein glycation through a cross-linking process. MGO has been involved in the pathogenesis of type 2 diabetes and several other age-related chronic inflammatory diseases (Schalkwijk & Stehouwer, 2020; de Oliveira et al., 2020). The discovery of MGO inhibitors from natural phytochemicals has received much attention. Shin et al. (2018) reported the MGO-scavenging activity of three natural compounds: (-)-epigallocatechin (EGC), (-)-epigallocatechin-3-gallate (EGCG), and quercetin, using the BSA-MGO assay system by measuring the fluorescence intensity at ex340/em420 nm. The results confirmed strong inhibitory activity against the formation of AGEs in a dose-dependent manner. The results of this present study agree with those reported by Shin et al. (2018) in that quercetin is a strong MGO trapper. Gallic acid, a natural polyphenol, is known for its antioxidant properties and can effectively scavenge free radicals and inhibit MGO (Liu et al., 2022). In the control tube, MGO-BSA remarkably increased. However, when treated with the extracts, the MGO-BSA fluorescence intensity was lower. As shown in Table 3, all plant extracts (1 μ g/mL) could inhibit the MGO-BSA formation by more than 50%, except *H. sabdariffa* ($47.52 \pm 0.97\%$), while AMG and gallic acid exhibit less than 50%.

Dityrosine is a compound formed through the oxidation of tyrosine amino acid residues. The presence of dityrosine can be used to assess oxidative stress and protein damage. When protein damage occurs due to ROS, advanced oxidation protein products can lead to protein aggregation, cross-linking, and fragmentation (Mengstie et al., 2022). Dityrosine cross-links in proteins and peptides are clinical markers of oxidative stress, aging, and neurodegenerative diseases (Maina et al., 2023). Leaf extracts showing high inhibition of dityrosine might be due to their antioxidant capacity to reduce ROS, which is the key factor in dityrosine formation. As shown in Table 3, *C. formosum* demonstrated the highest dityrosine inhibition ($98.59 \pm 0.26\%$), whereas *H. sabdariffa* ($47.68 \pm 2.52\%$) possessed the lowest activity.

Pentosidine is a highly cross-linked and fluorescent AGE derived from one molecule of arginine and one of lysine linked together in an imidazo-pyridinium structure

from the Maillard reaction. Pentosidine can form from either glucose or ribose, requiring oxidation; thus, it is both an AGE and a glycoxidation product. Nowadays, pentosidine is a potential biomarker for various conditions like diabetes, kidney dysfunction, and age-related diseases. Due to its association with diabetic complications, pentosidine is considered to be a potential biomarker for monitoring disease progression and assessing the effectiveness of interventions (Morales et al., 2011). All the test samples exhibited a high potential to pentosidine inhibition, ranging from $71.50 \pm 0.71\%$ to $99.50 \pm 0.14\%$.

It should be noted that *H. sabdariffa* leaf did not show antiglycation activity at day 7 but suppressed MGO-BSA, dityrosine, and pentosidine formation with the percentage inhibition of $47.52 \pm 0.97\%$, $47.68 \pm 2.52\%$, and $78.46 \pm 0.73\%$, respectively. *M. alba* and *S. antisepticum* possessed low total inhibition of AGEs but revealed higher potential for inhibiting MGO-BSA, dityrosine, and pentosidine formation. The *A. indica* flower produced similar results to this group of plants in all parameters.

3.6 Determination of tryptophan fluorescence quenching

In this experiment, the fluorescence quenching of BSA caused by glucose and plant extracts was evaluated. BSA has three fluorophores, namely tryptophan, tyrosine, and phenylalanine, whereas tryptophan mainly responds to the fluorescence intensity. BSA contains two tryptophan residues: Trp-212 is located within a hydrophobic binding pocket of the protein, and Trp-134 is located on the surface of the molecule (Han et al., 2012). Tryptophan possesses intrinsic fluorescence and is used to study the conformation of proteins after binding with other small molecules. The maximum emission wavelength (λ_{max}) was 364 nm when excited at 280 nm. In this step, the focus is on the fluorescence quenching of glycosylated BSA incubated with plant extracts. It could be clearly observed that the fluorescence intensity of BSA decreased after glycation, indicating changes in the protein micro-environment upon glucose attachment (Figure 4 (A)). This process refers to quenching, a phenomenon where the fluorescence intensity of BSA decreases due to interactions with other molecules. The quenching mechanism can involve various interactions, including hydrophobic interactions, hydrogen bonds, or van der Waals forces. Tryptophan fluorescence quenching is a powerful biophysical technique used to study protein structure, conformational changes, and intermolecular interactions. A reduction in fluorescence intensity often indicates alterations in its local environment caused by conformational transitions, ligand binding, subunit association, or protein denaturation (Dos Santos Rodrigues et al., 2023). Various studies on the quenching of BSA by small molecules have been reported. BSA or HSA can interact with a variety of molecules, such as ascorbic acid (Song et al., 2023), gallic acid (Khatun et al., 2018), and active pharmaceutical ingredients (APIs). Quercetin and most natural compounds are likewise BSA quenchers.

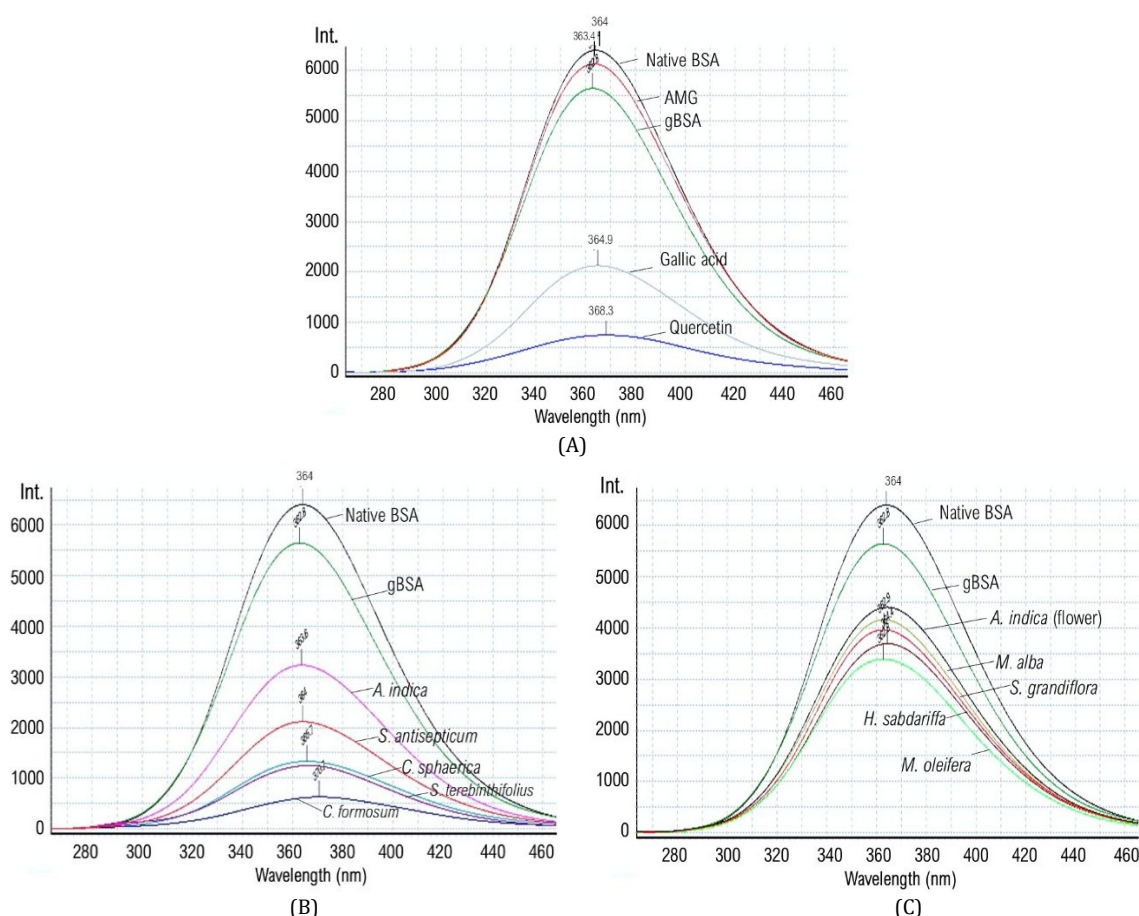


Figure 4. Tryptophan fluorescence quenching spectra of native BSA and BSA incubated at 40 °C for 7 days with 250 mM glucose (gBSA) in the absence and presence of different pure chemicals (1 mM) or plant extracts (1 µg/mL): (A) aminoguanidine HCl (AMG), gallic acid, and quercetin; (B) high total phenolic content extracts (*A. indica* (leaf), *S. antisepticum*, *S. sphaerica*, *S. terebinthifolius*, and *S. formosum*), and (C) low total phenolic content extracts (*A. indica* (flower), *M. alba*, *S. grandiflora*, *H. sabdariffa*, and *M. oleifera*)

Note: The tryptophan fluorescence quenching was detected using excitation at 280 nm.

According to Figure 4 (A), the strongest fluorescence intensity was found in native BSA, whereas the intensity of glycated BSA decreased. In the presence of AMG, the intensity was higher than gBSA due to the antiglycation effect. AMG also exhibited quenching properties, altering the secondary structure of BSA; hence protecting the integrity and functionality of proteins from glycation (Khan et al., 2021). In the presence of plant extracts (Figure 4 (B)), the fluorescence intensity of BSA incubated with high total phenolic content extracts decreases in a similar manner in quercetin and gallic acid. Quercetin and *C. formosum*, which had the highest AGEs inhibition, revealed the highest quenching effect, and the conformation of BSA was observed from the occurrence of a red shift in the fluorescence peak. Figure 4 (C) demonstrates a lesser quenching effect on the extracts containing lower amounts of total phenolic content, including *A. indica* flower extract. *M. oleifera* leaf extract showed a blue shift conformation due to an increase in hydrophobicity around tryptophan after binding. Plant extracts with higher equivalent amounts of total phenolic content and total tannin content performed a higher degree of quenching. This indicated that phytochemical

compounds in the extract can interact or form a complex with tryptophan residue or a nearby location, leading to a decrease in the ability to emit light. Quenching may be one mechanism that inhibits glycation by competitively binding to BSA's binding site, thereby forming a complex or blocking glucose attachment (Deng et al., 2023). Figure 5 illustrates the proposed mechanism by which plant extracts alter the conformation of BSA, potentially inhibiting glycation by blocking glucose binding sites. These extracts also exert antioxidant effects that help prevent the formation of AGEs. The strength and nature of these interactions depend on the specific structural features of the phenolic compounds, particularly the number and size of their aromatic rings.

3.7 Pearson correlation

Pearson correlation analysis showed that antioxidant capacity strongly correlated with total phenolic content (TPC), $r = 0.8600$ ($p = 0.0001$), and total tannin content (TT), $r = 0.9582$ ($p = 0.0001$), and exhibited moderate correlation with total flavonoid content (TFC), $r = 0.4015$ ($p = 0.0279$)

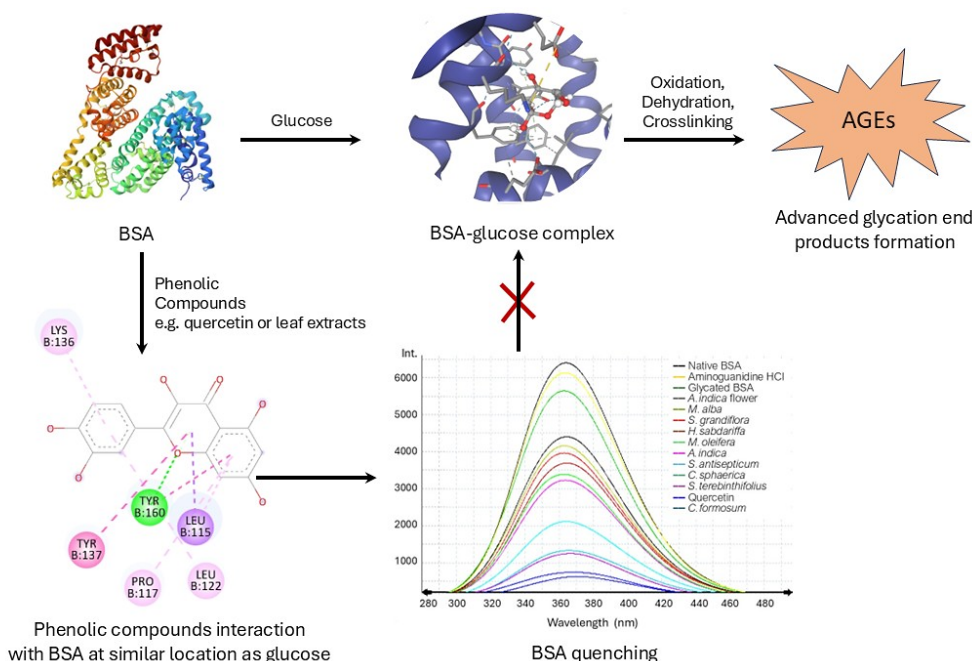


Figure 5. Schematic illustration of the primary non-covalent interactions between phenolic compounds and bovine serum albumin (BSA); hydrogen bonding occurs between the hydroxyl groups of polyphenols and the oxygen atoms present in the peptide backbone of BSA; hydrophobic interactions typically occur between the aromatic ring structures of polyphenols and the hydrophobic amino acid residues of BSA

Table 4. Pearson correlation of total phenolic, total flavonoid, and total tannin contents with antioxidant capacity (mg AAE/g) and antiglycation activities (% inhibition)

	Correlation (r)				
	Antioxidant	Total AGEs	MGO-BSA	Dityrosine	Pentosidine
TPC	0.8600** (<i>p</i> = 0.0001)	0.4758* (<i>p</i> = 0.0079)	0.6659** (<i>p</i> = 0.0001)	0.7017** (<i>p</i> = 0.0001)	0.6405** (<i>p</i> = 0.0001)
TFC	0.4015* (<i>p</i> = 0.0279)	0.4568* (<i>p</i> = 0.0112)	0.6526** (<i>p</i> = 0.0001)	0.6290** (<i>p</i> = 0.0002)	0.5907** (<i>p</i> = 0.0006)
TT	0.9582** (<i>p</i> = 0.0001)	0.3796* (<i>p</i> = 0.0385)	0.5091* (<i>p</i> = 0.0041)	0.5659* (<i>p</i> = 0.0011)	0.5091* (<i>p</i> = 0.0041)

Note: * Correlation is statistically significant at *p* < 0.05 and ** at *p* < 0.001

According to the data, MGO-BSA, dityrosine, and pentosidine inhibition activities strongly correlated with TPC (*r* = 0.6405 to 0.7017, *p* < 0.001) and TFC (*r* = 0.5907 to 0.6526, *p* < 0.001) while having less correlation with TT (*r* = 0.5091 to 0.5659, *p* < 0.005). For total AGEs or vesperlysine-like AGEs' inhibition, TPC, TFC, and TT exhibited less correlation compared to MGO-BSA, dityrosine, and pentosidine, thus indicating that leaf extracts positively inhibit both early glycated products as well as AGEs (vesperlysine and pentosidine). The phytochemicals strongly responding to antiglycation were phenolic, flavonoid, and tannin compounds. In this study, four leaves, namely *C. sphaerica*, *C. formosum*, *S. terebinthifolius*, and *S. antisepticum*, were tannin-rich, aromatic leaves, contributing to their unique taste and pleasant smell. The high TT content of these leaves might be the reason for lower fluorescence intensity (quenching) and remarkably high antiglycation activity. These plants might be candidates for nutraceutical, functional food, or cosmetic purposes.

4. CONCLUSION

The ethanol extracted from the nine leaves of edible plants demonstrated high-potential antiglycation *in vitro*. The determination of intrinsic tryptophan fluorescence showed that the probable activity mechanisms might come from the molecular interactions of phytochemicals present in the plant with BSA, at least near the tryptophan residue. The insertion of phytochemical molecules into the inner part of BSA increases the hydrophobicity and reduces the interaction between glucose molecules and BSA. This study establishes the usefulness of the nine plants for their therapeutic potential against glycation-induced health disorders from endogenous or exogenous sources. Plants with both high antiglycation and high antioxidant capacity are potential bioactive compounds in the treatment of diabetes. *C. formosum* leaf extracts exhibited the strongest antiglycation activity in all types of measured parameters, while *A. indica* flower extract offered less antiglycation activity compared to *A. indica* leaf extract, despite its

remarkably high impact. These two edible parts are the most familiar in Thai cuisine. Although these edible leaves have a variety of TPC, TFC, and TT content, they demonstrate at least one promising antiglycation activity and have the potential to be safe and effective alternatives for health promoters. The mechanisms underlying the antiglycation activity and therapeutic potential of each individual plant extract require further investigation. Future research, particularly *in vivo* studies, is essential to confirm the extract's biological efficacy and safety. In addition, formulation development could enhance the stability and bioavailability of the extract, thereby facilitating its potential in clinical application.

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AUTHOR CONTRIBUTIONS

N.T. and S.C. designed the study. N.T., K.T. and S.C. conducted the experiments and analyzed the experimental data. N.T. and P.C. performed statistical analysis. S.C. wrote the manuscript with input of all authors. All authors revised and approved the final manuscript.

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