Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 8868031, 12 pages
https://doi.org/10.1155/2023/8868031

Research Article

Anti-Glycation, Anti-β-Amyloid Aggregation, and Antioxidant Effect of *Cassia* Seed-Derived Secondary Metabolites

Pradeep Paudel,[1] **Ritu Prajapati**,[2] **Hyun Jung Lim**,[3] **Srijan Shrestha**,[4] **Su Hui Seong**,[5] **Hyun Ah Jung**,[6] **and Jae Sue Choi**[3,7]

[1]*National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Oxford, MS 38677, USA*
[2]*Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA*
[3]*Institute of Fisheries Sciences, Pukyong National University, Busan 46041, Republic of Korea*
[4]*Discipline of Pharmacology, School of Biomedicine, Faculty of Health Sciences, The University of Adelaide, Adelaide 5005, SA, Australia*
[5]*Division of Natural Products Research, Honam National Institute of Biological Resource, Mokpo 58762, Republic of Korea*
[6]*Department of Food Science and Human Nutrition, Jeonbuk National University, Jeonju 54896, Republic of Korea*
[7]*Department of Food and Life Science, Pukyong National University, Busan 48513, Republic of Korea*

Correspondence should be addressed to Hyun Ah Jung; jungha@jbnu.ac.kr and Jae Sue Choi; choijs@pknu.ac.kr

Received 11 May 2023; Revised 6 September 2023; Accepted 29 September 2023; Published 21 October 2023

Academic Editor: Yu Fu

Aggregation and non-enzymatic glycation of amyloidogenic peptides, amyloid-beta (Aβ), and insulin are key features of neuropathology. In this study, we evaluate the anti-glycation effect of Cassia seed-derived secondary metabolites on human insulin and bovine serum albumin, as well as their anti-Aβ aggregation and antioxidant effects using in vitro spectrofluorometric method, thioflavin T fluorescence, and peroxynitrite ($ONOO^-$) scavenging assay. Furthermore, molecular docking simulation was performed to investigate the binding characteristics of test compounds and Aβ42 peptide. Among 38 compounds, anthraquinones **9**–**12** and **14**–**18**; naphthopyrones **24**, **25**, **27**, **and 33**–**36**; and **38** from the naphthalenes and naphthalenic lactone groups showed moderate-to-good inhibition of AGE formation with IC_{50} values ranging from 7.52 ± 1.19 to $155.86 \pm 0.79\,\mu$M. Likewise, compounds **5**, **24**, **15**, **16**, **27**, and **20** showed good inhibition of D-ribose-mediated glycation of human insulin, with an IC_{50} value range of 46.37 ± 4.06 to $97.69 \pm 7.88\,\mu$M. In the thioflavin-T assay, compounds **8** and **12** showed promising inhibition of Aβ aggregation, comparable to that of the reference compound morin. Molecular docking simulations confirmed that these active compounds have strong potential to interact with Aβ42 peptides and interrupt their self-assembly and conformational transformation, thereby inhibiting Aβ42 aggregation. In addition, compounds **5**, **8**, **10**, **14**, and **35** scavenged $ONOO^-$ at low concentrations. Overall, Cassia compounds' anti-glycation, anti-Aβ aggregation, and antioxidant effects warrant further in vivo studies to evaluate their potential neuroprotective effects against comorbid AD and diabetes.

1. Introduction

The non-enzymatic glycation or oxidation of the amino ($-NH_2$) groups of amino acids, proteins, peptides, and hormones by reducing sugars results in a set of different products, the advanced glycation end products (AGEs), that assemble in various parts of the human body and increase the risk of diabetic complications, including neuropathy, nephropathy, and cardiovascular diseases. In addition to their role in diabetic complications, AGEs have also been implicated in the pathogenesis of Alzheimer's disease (AD) and other neurodegenerative diseases [1]. The presence of senile plaques and neurofibrillary tangles (NFTs) characterizes the pathology in AD, and amyloid β protein (Aβ) and

tau proteins are their major constituents. Glycation of tau protein enhances paired helical filament formation [2], and glycation of Aβ prolongs its half-life, which accelerates amyloid protein aggregation [3].

Similarly, glycation of insulin and proinsulin in the insulin-resistant diabetic state [4] and α-synuclein in Parkinson's disease (PD) [5] is well documented. Spheroproteins or normal globular proteins can be refolded into amyloid fibrils (a major component of proteinaceous plaques), comprising a cross-β structure, during glycation [6]. In addition, a highly reactive intermediate product, 3-deoxyglucosone, of the glycation reaction can reinforce the formation of intermolecular and intramolecular cross-links in AGE-modified protein monomers.

Glycation of proteins by glucose occurs via several pathways [7]: (a) through nucleophilic addition between an amino group of a protein and the carbonyl group of glucose to form a reversible Schiff base, (b) through oxidative degradation in the presence of reactive oxygen species (ROS), and (c) through the reaction of dicarbonyl compounds from glucose auto-oxidation with the amino groups of proteins. Hormones, peptides, and proteins are endogenous sources of AGEs that are responsible for disease development. A modern diet comprised of high-heat-processed meats, processed milk, and bakery products with enhanced flavor and taste is a major contributor to oxidative stress, inflammation, and liver fibrosis, along with several chronic disease states [8]. In regard to the role of AGEs in the development of diabetic complications and other diseases, various natural and synthetic compounds have been evaluated for their anti-glycation properties. Inhibiting any step of glycation and preventing the formation of intermediate and end products, scavenging of free radicals, detoxification of liver enzymes, etc. can help in the prevention of AGE formation and diabetic complications [9]. To date, numerous synthetic compounds with anti-glycation properties have been discovered including but not limited to urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analog [10], bis-Schiff bases of isatins [11], benzimidazole derivatives [12], oxindole derivatives [13], bergenin derivatives [14], and piroxicam derivatives [15]. However, due to fewer complications compared to synthetic compounds, natural compounds are considered a better treatment for inhibiting the glycation process and AGE formation. Therefore, there is an emerging interest in the discovery of new anti-glycation agents from natural sources. With this aim, we investigate *Cassia obtusifolia* Linn seeds-derived secondary metabolites.

Cassia obtusifolia Linn seed is a traditional Chinese medicine that is well known for its anti-inflammatory and antioxidant properties. Cassia seed is consumed as brewed tea in South Korea. Cassia seed extract has been reported to have numerous biological activities, including hypolipidemic [16, 17], antifungal [18], hypotensive [19], antioxidant [20], and neuroprotective [21] effects. Similarly, secondary metabolites from Cassia seeds have larvicidal [22], antioxidant [23], anti-AD [24], hepatoprotective [25], antidiabetic [26], antihypertensive [27], comorbid diabetes and depression [28] and antimutagenic effect [29]. However, there are a limited number of studies on the anti-glycation properties of Cassia seed-derived metabolites. A few numbers of naphthopyrone glycosides [30] and anthraquinones [31] have previously been reported to have an inhibitory effect on BSA-glycation. This study reports the anti-glycation properties of other Cassia compounds in human insulin and bovine serum albumin, as well as anti-Aβ aggregation and antioxidant effect. To our knowledge, this is the first report of the anti-glycation and insulin-glycation inhibition properties of these metabolites.

2. Materials and Methods

2.1. Chemicals and Reagents. Bovine serum albumin (BSA), L-penicillamine, D-(−)-fructose, D-(+)-glucose, D-(−)-ribose, human insulin, aminoguanidine hydrochloride, dihydrorhodamine (DHR) 123, and diethylenetriamine-pentaacetic acid (DTPA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The Aβ42 peptide was purchased from Bachem AG (Bubendorf, Switzerland). Peroxynitrite was obtained from Calbiochem/Merck Millipore (Darmstadt, Germany). Solvents for column chromatography were of reagent grade and were used as received from commercial sources.

2.2. Isolation of Compounds. Details on plant material, extraction, fractionation, and isolation have been described in our recent work [32]. Chemical structures of isolated compounds are shown in Figure 1.

2.3. Bovine Serum Albumin Glycation Inhibition Assay. Following some modifications in the spectrofluorometric method previously described by Vinson and Howard [33], the inhibitory potential of isolated compounds in AGE formation was evaluated. At first, the AGE reaction mixture was prepared by mixing 10 mg/ml BSA in 50 mmol/L sodium phosphate buffer (pH 7.4), 0.2 M fructose, and 0.2 M glucose. To prevent bacterial growth, the reaction mixture was maintained in 0.02% sodium azide. Subsequently, 950 μL of reaction buffer was mixed with 50 μL of the test sample (2.5 μM to 250 μM) solution in vials and incubated at 37°C for 7 days. Color controls were prepared similarly and incubated at 4°C for 7 days. The inhibition percentage was calculated by measuring the fluorescence intensity of the reaction product at 450 nm after excitation at 350 nm in a microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA). The inhibition result was validated with aminoguanidine HCl as a positive control.

2.4. Human Insulin Glycation Inhibition Assay. The inhibition potential of test compounds on insulin glycation was evaluated according to a recently described method [34], with slight modifications. First, monomeric insulin was obtained by dissolving human insulin in ultra-pure water at pH 2.0. Then, the monomeric insulin solution was neutralized to pH 7.0 and kept in phosphate buffer (50 mM; pH 7.0). To obtain a glycated insulin control, 10 μL of monomeric human insulin (final concentration:

0.5 mg/mL), 10 μL of 0.5 M D-ribose in phosphate buffer (50 mM; pH 7.0), and 10 μL of ultra-pure water were mixed, filtered through 0.2 μm filter, and incubated at 37°C for 7 days. The anti-glycation properties of test compounds were evaluated by adding 10 μL of the test sample (2.5 μM to 250 μM; replacing 10 μL water from the control) to a mixture of insulin and D-ribose and incubating at 37°C for 7 days. For the protein control, human insulin was incubated with buffer only. Then, the fluorescence signal was measured at λ_{ex} 320 nm/λ_{em} 410 nm in a microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA, USA).

2.5. Anti-Aggregation Assay. ThT is known to exhibit fluorescence upon binding to the β-sheet structure [35]. We used ThT analysis to investigate the effect of test compounds on the aggregation of Aβ that undergoes structural conversion into a β-sheet-rich fiber. For Aβ aggregation, we added 3rd distilled water to synthetic Aβ42 to produce Aβ42 at a concentration of 25 μM. The Aβ peptide chemically treated with aqueous ammonia, purchased from Bachem AG (Bubendorf, Switzerland), is monomeric and has no fluorescence when measured by the ThT assay. We used a 25 μM concentration that was obtained from 50% toxicity among different concentrations in the cell viability test. The Aβ42 peptide, purchased from Bachem AG (Bubendorf, Switzerland), was dissolved in 0.1 M aqueous ammonia. To investigate the effects of Cassia compounds on the aggregation of Aβ, ThT assays with non-aggregated Aβ42 were performed. ThT assays were carried out in black polypropylene 96-well plates (SPL Life Science, Republic of Korea). For Aβself-aggregation, triple distilled water was added to the Aβ42 stock until it reached a concentration of 25 μM. Various concentrations of Cassia compounds (2.5, 25, and 250 μM) and the positive control, morin (100 μM), were added together with a ThT solution (15 μM in 50 mM glycine buffer, pH 8.9) into the 25 μM Aβ42 solution. All solutes used in the experiment were well soluble in the solvent. They were then incubated for 3 h at 37°C (Figure 2). Finally, the ThT fluorescence intensity was measured at excitation wavelengths of 440 nm and emission wavelengths of 484 nm using a SpectraMax iD3 Multi-Mode Microplate Reader (Molecular Devices, San Jose, CA, USA). Experiments were performed in triplicate.

2.6. Molecular Docking Simulation. We used AutoDock 4.2 to investigate the binding characteristics of test compounds and Aβ42 peptide. To determine the receptor structures, we obtained the three-dimensional structures of Aβ42 peptides from the Protein Data Bank (PDB code: Aβ42: 1Z0Q and 2BEG). Two- and three-dimensional structures of the test compounds and morin were obtained from the PubChem database, and energy minimization and ligand structure conversion to the PDB file format were performed using Chimera 1.15 (https://www.cgl.ucsf.edu/chimera). For molecular docking purposes, we prepared receptors and ligands using AutoDockTools, whereas to conduct molecular docking, we applied the prepared macromolecules and ligands using AutoDockTools [36]. In the case of the monomer structures of Aβ, we used Autogrid to generate grid parameters centered on the reference ligand and created a grid box of $60 \times 60 \times 60$ Å. In the case of Aβ42 pentamer (2BEG), the grid parameters were generated using the mean coordinates of the hydrophobic core (16KLVFFA 21) and C-terminal (29GAIIGLMVGGVVIA42) segments, and grid boxes of $90 \times 90 \times 90$ Å were set at the established coordinates using Autogrid. One hundred Lamarckian generic algorithms were run for docking, and the other docking parameters were set to default values. The docking results were analyzed using AutoDockTools and visualized using Discovery Studio (v17.2, Accelrys, San Diego, CA, USA). The docking pose with the lowest binding energy and the highest number of cluster populations was selected for the analysis of docking results.

2.7. Peroxynitrite ($ONOO^-$) Scavenging Assay. ONOO-scavenging activity was assessed using the modified Kooy's method, which involves the monitoring of highly fluorescent rhodamine 123, which is rapidly produced from non-fluorescent DHR 123 in the presence of $ONOO^-$ [37]. In brief, the rhodamine buffer (pH 7.4) consisted of 50 mM sodium phosphate dibasic, 50 mM sodium phosphate monobasic, 90 mM sodium chloride, 5 mM potassium chloride, and 100 μM DTPA. The final DHR 123 concentration was 5 μM. The assay buffer was prepared before use and placed on ice. Test samples were dissolved in 10% DMSO (final conc., 100 μM). The background and final fluorescent intensities were measured 5 min after treatment with and without the addition of authentic $ONOO^-$ (10 μM), dissolved in 0.3 N sodium hydroxide. The fluorescence intensity of the oxidized DHR 123 was evaluated using a microplate spectrofluorometer (Bio-Tek Instruments Inc., Winooski, VT, USA) at excitation and emission wavelengths of 480 and 530 nm, respectively. The values of $ONOO^-$ scavenging activity were calculated as the final fluorescence intensity minus the background fluorescence, via the detection of DHR 123 oxidation. L-Penicillamine was used as the positive control.

2.8. Statistical Analysis. All statistical analyses were conducted using the GraphPad Prism 7.0 software (GraphPad Software, La Jolla, CA, USA). All statistical data are displayed as mean ± standard error of the mean (SEM). An independent *t*-test was used to evaluate the normality of the data. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Inhibition of D-Glucose/D-Fructose-Mediated Glycation of Bovine Serum Albumin. The protective effect of anthraquinones, naphthopyrones, naphthalenes, and naphthalenic lactones (Figure 1) isolated from Cassia seeds against protein glycation was evaluated in vitro, and the results are tabulated in Table 1. As shown in the table, most of the anthraquinones showed good inhibition potential on AGE formation.

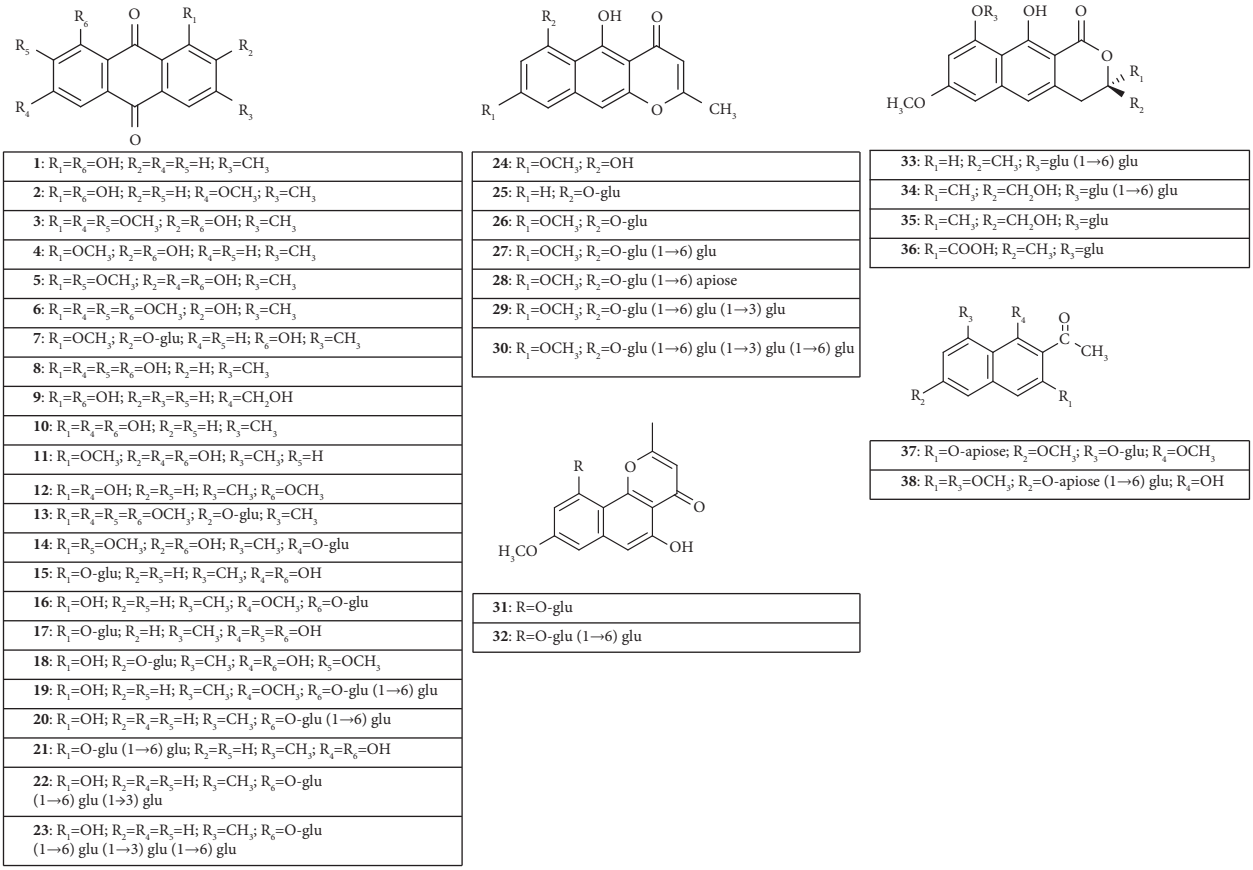

Figure 1: Chemical structures of compounds isolated from *Cassia obtusifolia* Linn seeds.

Among the active anthraquinone aglycones, **8** (IC_{50}; $11.50 \pm 0.07\,\mu M$) was the most active, followed by **11** (IC_{50}; $86.29 \pm 7.09\,\mu M$) and **12** (IC_{50}; $118.12 \pm 3.11\,\mu M$). Aglycones **9**, **10**, and **5** had a similar effect (IC_{50} values approx. $170\,\mu M$), while **6** was slightly weaker (IC_{50}; $189.61 \pm 1.70\,\mu M$). However, aglycones **1**–**4** were ineffective up to $200\,\mu M$ under the given experimental conditions.

Similarly, among anthraquinone glucosides, only single glucose-bearing anthraquinones (**14**–**18**, except **7** and **13**) were active. The most potent among these compounds was **18** (IC_{50}; $32.51 \pm 1.40\,\mu M$). Other glucosides **14**–**17** had an IC_{50} in the range of 105 to $155\,\mu M$. Anthraquinone glycosides with two or more glucose moieties (**19**–**23**) were ineffective up to $200\,\mu M$.

From the naphthopyrone series, only **24**, **25**, **27,** and **33**–**36** were active. The most potent compound in this series was **36** (IC_{50}; $7.52 \pm 1.19\,\mu M$), followed by **38** (IC_{50}; $11.63 \pm 0.12\,\mu M$), **37** (IC_{50}; $23.40 \pm 1.05\,\mu M$), **27** (IC_{50}; $38.89 \pm 2.05\,\mu M$), **35** (IC_{50}; $40.36 \pm 1.36\,\mu M$), **24** (IC_{50}; $42.58 \pm 1.79\,\mu M$), and **25** (IC_{50}; $101.37 \pm 4.72\,\mu M$). Other glycosides **26** and **28**–**32** showed no observable effect up to $200\,\mu M$.

Similarly, between the two compounds from naphthalenes and naphthalenic lactone group, only **38** showed a moderate effect, with an IC_{50} value of $89.03 \pm 3.08\,\mu M$. Interestingly, **33** was ineffective up to $200\,\mu M$. The overall results of this in vitro anti-glycation assay revealed that naphthopyrones from Cassia seeds are promising inhibitors of protein glycation. Furthermore, all of the active compounds had better activity than the positive control, aminoguanidine HCl (IC_{50}; $590.94 \pm 2.81\,\mu M$).

3.2. Inhibition of D-Ribose-Mediated Glycation of Human Insulin. Next, we evaluated the effect of Cassia compounds on D-ribose-mediated glycation of human insulin. As shown in Table 1, among twenty-three anthraquinones, only four (**5**, **15**, **16**, and **20**) showed moderate inhibition of insulin glycation with an IC_{50} value range of 46.37 ± 1.19 to $83.23 \pm 0.38\,\mu M$. Rubrofusarin (**24**) and its gentiobioside (**27**) also showed moderate inhibitory activity with IC_{50} values of 87.03 ± 3.93 and $97.69 \pm 7.88\,\mu M$, respectively. None of the naphthalene and naphthalenic lactones exhibited inhibitory activity.

3.3. Inhibitory Effect on Aβ42 Self-Aggregation. Some anthraquinones, naphthopyrone, and flavonoids have been reported to inhibit the formation of Aβ fibrils in a ThT study [38–40]. In this study, the effect of aurantio-obtusin (**5**), chryso-obtusin (**6**), aloe-emodin (**9**), 2-hydroxyemodin (**8**), questin (**12**), glucoaurantio-obtusin (**14**) and naphthopyrones – nor-rubrofusarin glucoside (cassiaside, **25**), rubrofusarin apiosylglucoside (**28**), and toralactone gentiobioside (**33**) on Aβ42 aggregation was evaluated. We observed that various concentrations of these

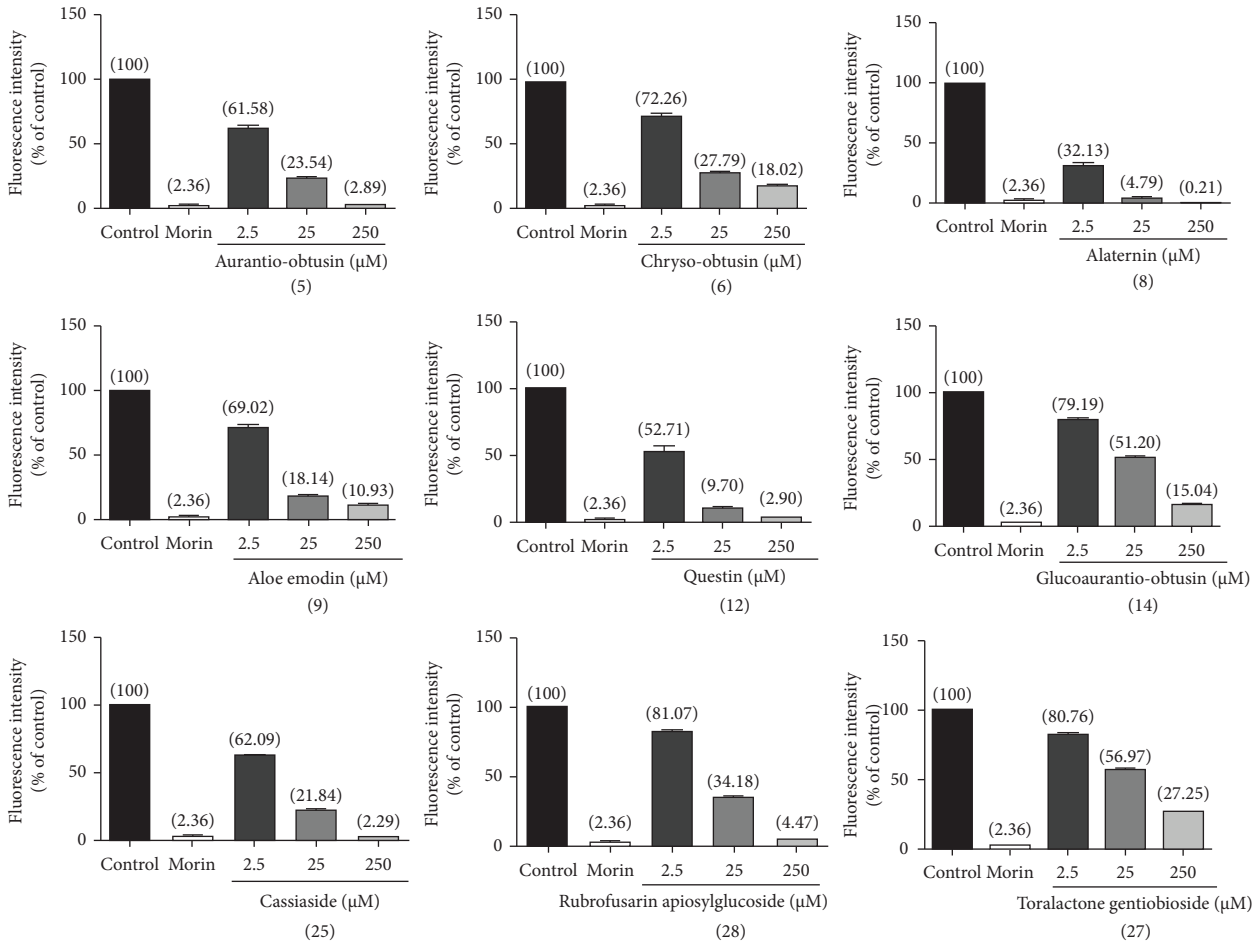

Figure 2: Anti-aggregation effect of different kinds of Cassia compounds on Aβ42. The molar ratios of Aβ : Cassia compounds are 10 : 1 (2.5 μM), 1 : 1 (25 μM), and 1 : 10 (250 μM). The vehicle group was treated with 3rd distilled water. Morin (100 μM) was used as a positive control as an Aβ aggregation inhibitor. Values in parentheses are percentages of the control value. Numbers below the compound name in each graph represent the assigned compound number.

Table 1: Anti-glycation effect of major anthraquinones and naphthopyrones from *Cassia obtusifolia* Linn seeds.

Compounds	BSA	Human insulin
	IC_{50} (μM)[a]	
Anthraquinones		
Chrysophanol (**1**)	>200	ND
Physcion (**2**)	>200	ND
Obtusin (**3**)	>200	ND
Obtusifolin (**4**)	>200	ND
Aurantio-obtusin (**5**)	166.33 ± 0.83	55.69 ± 1.19
Chryso-obtusin (**6**)	189.61 ± 1.70	135.26 ± 2.98
Obtusifolin 2-glucoside (**7**)	>200	>200
7-Hydroxyemodin (alaternin) (**8**)	11.50 ± 0.07	>200
Aloe-emodin (**9**)	169.79 ± 2.97	122.27 ± 5.49
Emodin (**10**)	169.56 ± 1.09	>200
2-Hydroxyemodin 1-methyl ether (**11**)	86.29 ± 7.09	140.82 ± 2.56
Questin (emodin-8-methyl ether) (**12**)	118.12 ± 3.11	194.80 ± 5.01
Chryso-obtusin 2-glucoside (**13**)	>200	>200
Glucoaurantio-obtusin (**14**)	155.86 ± 0.79	146.60 ± 4.69
Emodin 1-O-β-D-glucopyranoside (**15**)	105.61 ± 6.32	67.45 ± 3.02
Physcion 8-O-β-D-glucopyranoside (**16**)	120.64 ± 2.97	83.23 ± 0.38
Alaternin 1-O-β-D-glucopyranoside (**17**)	113.49 ± 3.05	159.12 ± 9.61
1-Desmethylaurantio-obtusin 2-O-β-D-glucopyranoside (**18**)	32.51 ± 1.40	>200
Physcion 8-O-β-gentiobioside (**19**)	>200	>200
Chrysophanol 1-O-β-gentiobioside (**20**)	>200	46.37 ± 4.06

Table 1: Continued.

Compounds	BSA	Human insulin
	IC_{50} (μM)[a]	
Emodin 1-O-β-gentiobioside (**21**)	>200	>200
Chrysophanol triglucoside (**22**)	>200	>200
Chrysophanol tetraglucoside (**23**)	>200	>200
Naphthopyrones		
Rubrofusarin (**24**)	42.58 ± 1.79	87.03 ± 3.93
Cassiaside (**25**)	101.37 ± 4.72	>200
Rubrofusarin 6-O-β-D-glucopyranoside (**26**)	>200	>200
Rubrofusarin 6-O-β-D-gentiobioside (**27**)	38.89 ± 2.05	97.69 ± 7.88
Rubrofusarin 6-O-β-D-apiofuranosyl-(1⟶6)-O-β-D-glucopyranoside (**28**)	>200	175.38 ± 6.62
Rubrofusarin triglucoside (**29**)	>200	128.35 ± 4.39
Rubrofusarin tetraglucoside (cassiaside B2) (**30**)	>200	>200
Demethylflavesperone 10-O-β-D-glucopyranoside (**31**)	>200	>200
Isorubrofusarin gentiobioside (**32**)	>200	163.79 ± 5.97
Toralactone 9-O-β-gentiobioside (**33**)	40.36 ± 1.36	>200
Cassialactone 9-O-β-gentiobioside (**34**)	7.52 ± 1.19	>200
(3R)-Cassialactone 9-O-β-D-glucopyranoside (**35**)	23.40 ± 1.05	>200
(3S)-9,10-Dihydroxy-7-methoxy-3-methyl-1-oxo-3,4-dihydro-1H-benzo[g] isochromene-3-carboxylic acid 9-O-β-D-glucopyranoside (**36**)	11.63 ± 0.12	>200
Naphthalenes and Naphthalenic lactones		
Cassitoroside (**37**)	>200	>200
1-Hydroxyl-2-acetyl-3,8-dimethoxy-naphthalene-6-O-β-D-apiofuranosyl-(1⟶2)-β-D-glucopyranoside (**38**)	89.03 ± 3.08	>200
Aminoguanidine[b]	590.94 ± 2.81	—

[a]The 50% inhibitory concentration (IC_{50}) values (μM) were calculated from a log dose inhibition curve and are expressed as mean ± SEM of triplicate experiments. [b]Positive control.

compounds (2.5, 25, and 250 μM) significantly inhibited Aβself-aggregation in a dose-dependent manner. Morin, which was used as a positive control, significantly reduced Aβself-aggregation (Figure 2).

3.4. In Silico Molecular Docking Simulation of Aβ Monomers. We conducted an *in silico* study to elucidate the mechanism underlying the anti-aggregation effects of different kinds of Cassia compounds on Aβ42. There are two types of Aβ monomers: α-helical and β-sheet. Therefore, we performed a molecular docking study to evaluate the binding between these Cassia compounds and the two types of Aβ chains (Tables S1–S3; Figures S1–S3). Since some of the anthraquinones, naphthopyrones, and flavonoids have already been reported for inhibition of Aβ aggregation [38–40], we chose unreported compounds for Aβ42 aggregation assay and predicted their binding affinities via docking study. Cassia compounds interacted with the Aβ α-helical monomer (1Z0Q) and the β-sheet monomer and pentamers (2BEG) with a binding affinity of about −4.00 to −5.00 kcal/mol, involving hydrogen bonds and hydrophobic and electrostatic interactions (Tables S1–S3; Figures S1–S3). Morin, a reference ligand, and 1Z0Q Aβ formed a complex with a binding affinity of −4.66 kcal/mol through three hydrogen bond interactions (Gln15, Glu11, Glu22) and a π-anion interaction with Glu22 (Table S1 and Figure S1). Morin interacted with the 2BEG Aβ pentamer via three hydrogen bonds ($Leu17^{C,E}$, $Phe19^{D}$) and six hydrophobic bonds with residues $Leu17^{E}$ (π-alkyl), $Leu17^{D}$ (π-σ, π-alkyl), $Phe19^{C}$ (π-π T-shaped), $Val40^{C}$ (π-σ, π-alkyl), and $Val40^{D,E}$ (π-alkyl) with a binding affinity of −7.42 kcal/mol (Table S3 and Figure S3). Anthraquinones and naphthopyrone glycosides from Cassia seeds bound to the α-helical and β-sheet monomers in a similar manner as the reference compound morin (Tables S1 and S2; Figures S1 and S2), demonstrating an anti-aggregation effect on Aβ42, which was verified with the ThT assay. We also conducted a molecular docking study on Cassia compounds and the β-sheet pentamer (2BEG) to determine the interaction of the test compounds with Aβ aggregates. We found that these compounds conjugated with Aβ42 β-sheet pentamers with a binding affinity of −6.39 to −9.02 kcal/mol via a hydrogen bond interaction, hydrophobic interactions, and a π-lone pair interaction (Table S3 and Figure S3).

3.5. Peroxynitrite ($ONOO^-$) Scavenging Activity. In the case of the ONOO-radical scavenging assay, compounds **8** and **33** showed strong scavenging activity with EC_{50} values of 0.83 ± 0.03 and 2.05 ± 0.04 μM, respectively (Table 2). Compounds **5** and **14** also showed potent activity with EC_{50} values of 4.33 ± 0.12 and 9.34 ± 0.86 μM, respectively. Interestingly, the $ONOO^-$ radical scavenging activity of compound **8** was two-fold higher than that of the positive control, L-penicillamine (IC_{50} = 1.61 ± 0.09 μM). Compound **33** showed a similar scavenging activity as L-penicillamine. Other test compounds (Table 2) showed moderate to mild scavenging effects. Altogether, our study demonstrates that compounds **8**, **33**, **5**, and **14**, in descending order, are promising antioxidants.

Table 2: Peroxynitrite scavenging activity of compounds isolated from *C. obtusifolia*.

Compound	Concentration (μgmL^{-1})[a]	Inhibition (%) (mean ± SD)	IC_{50}[b]	
			μgmL^{-1}	μM
Obtusifolin (**4**)	10	17.51 ± 8.02	38.99 ± 1.85	137.28 ± 2.45
	50	75.37 ± 4.34		
Aurantio-obtusin (**5**)	0.4	33.64 ± 3.88	1.43 ± 0.24	4.33 ± 0.12
	2	59.05 ± 3.57		
	10	71.72 ± 1.74		
	50	87.75 ± 1.67		
Obtusifolin 2-glucoside (**7**)	10	9.02 ± 3.93	50.68 ± 1.89	113.63 ± 3.14
	50	49.50 ± 2.73		
7-Hydroxyemodin (alaternin) (**8**)	0.08	29.60 ± 2.80	0.24 ± 0.02	0.83 ± 0.03
	0.4	69.31 ± 2.45		
	2	95.86 ± 0.84		
Emodin (**10**)	2	5.21 ± 7.83	14.83 ± 3.00	54.92 ± 1.22
	10	42.79 ± 5.02		
	50	96.14 ± 41.20		
2-Hydroxyemodin 1-methyl ether (**11**)	10	16.87 ± 7.29	30.78 ± 3.21	102.60 ± 7.11
	50	79.16 ± 2.42		
Questin (**12**)	10	2.65 ± 2.00	33.95 ± 0.50	119.54 ± 4.81
	50	85.21 ± 0.54		
Glucoaurantio-obtusin (**14**)	2	32.59 ± 8.05	4.60 ± 0.12	9.34 ± 0.86
	10	79.51 ± 0.26		
Chrysophanol triglucoside (**22**)	10	4.37 ± 2.29	59.27 ± 1.55	80.09 ± 0.97
	50	41.70 ± 0.74		
Toralactone 9-O-β-gentiobioside (**33**)	0.4	36.05 ± 1.59	1.22 ± 0.06	2.05 ± 0.04
	2	63.07 ± 0.38		
	10	82.92 ± 0.80		
	50	93.22 ± 0.50		
Penicillamine	0.4	56.10 ± 1.54	0.24 ± 0.04	1.61 ± 0.09
	2	82.92 ± 1.51		
	10	97.39 ± 1.60		

[a]Final compound concentration of the test samples was 0.4–50 μgmL^{-1}, which were dissolved in DMSO. [b]The concentrations producing 50% inhibition (IC_{50}, in μgmL^{-1} and μM) were calculated from the log dose inhibition curve and expressed as mean ± SEM of duplicate experiments.

4. Discussion

Advanced glycation end products (AGEs) are a heterogeneous class of molecules (hydroimidazolones, 3-deoxyglucosone derivatives, bis(lysyl)imidazolium cross-links, and monolysyl adducts) formed due to non-enzymatic protein glycation by glucose, which have detrimental effects on the normal physiology and functions of a biological system. The formation and accumulation rate of AGEs is elevated in diabetes, renal failure, and aging. Considerable evidence links accelerated AGE formation with hyperglycemia, which leads to the development of long-term vascular complications due to subsequent accumulation in vessel wall proteins [41].

The ability of insulin to regulate plasma glucose homeostasis is impaired by glycation. Once insulin is glycated, insulin receptors do not recognize the hormone, prolonging the half-life, which leads to hyperinsulinemia and insulin resistance [42]. A recent study by Iannuzzi et al. [43] demonstrated that D-ribose-induced insulin glycation affects endothelial cell viability through the mitochondrial pathways of apoptosis (caspase 9 and 3/7), intracellular ROS activation, and the transcription factor NF-kB activation. ROS is one signaling molecule for signal transduction of several receptors, including the receptor for advanced glycation end products (RAGE). The interaction of this receptor with AGEs causes oxidative stress by enhancing ROS generation and NF-kB activation.

AD and T2D share many pathophysiological features, including increased oxidative stress and amyloid aggregation [44], and cerebrovascular disease is one of the major complications of T2D. Of the various forms of insulin, a key hormone regulating glucose homeostasis, monomeric and dimeric forms are less stable than hexamers and tend to aggregate, forming amyloid fibrils [45, 46]. Pathological conditions related to insulin fibril formation can occur in diabetic patients. In addition, insulin is susceptible to non-enzymatic glycation under diabetic conditions. Accumulation of the end products of glycation, AGEs, is the main factor responsible for the development and progression of several diabetic complications, including nephropathy, retinopathy, and neuropathy [47]. In addition, increasing evidence suggests that depending on the glycating agent, glycation differentially affects the amyloid aggregation [48, 49]. Thus, the study of anti-amyloidogenic and anti-AGE agents to develop new potential therapeutic strategies

in amyloid-based neurodegenerative disease is increasing [50, 51].

In light of these considerations, we designed the current study to investigate the anti-glycation effect of Cassia seed-derived secondary metabolites in human insulin and bovine serum albumin, as well as their anti-Aβ aggregation and antioxidant effects.

Thioflavin T fluorescence enhancement indicates the structural changes and formation of fibrils as a result of glycation. Natural products have been shown to offer protection from structural changes caused by glycation [52]. In the Aβ aggregation assay, anthraquinones **5**, **8**, **9**, and **12** and naphthopyrone glycosides **25** and **28** showed promising inhibition of Aβself-aggregation in a dose-dependent manner. Several low-molecular-weight compounds have recently been discovered that can alter the Aβ-aggregation process. However, detailed descriptions of their interactions with oligomers and fibrils have so far been lacking. In a previous study, molecular dynamics simulations were used to investigate the effect of two relatively similar planar tricyclic compounds, 9,10-anthraquinone and anthracene, on the early stages of segmental Aβ-aggregation [39]. Simulations demonstrated that 9,10-anthraquinone hinders the heptapeptide segment H14QKLVFF20, a hydrophobic stretch that promotes Aβself-assembly more than anthracene. In particular, 9,10-anthraquinone intercalates into β-sheets because polar interactions between compounds and peptide backbones disrupt interchain hydrogen bonds and promote their failure. Recently, a similar study demonstrated that emodin, daunorubicin, and Adriamycin inhibit the aggregation of tau protein into paired helical filaments [53]. Likewise, carmine, an anthraquinone core attached to a glucose moiety, effectively reduced aggregation of the model 41 GCWMLY 46 peptide fragment (GDC6 peptide) in a dose-dependent manner [54]. Natural quinones remain an important source of novel anti-amyloid inhibitors. Emodin and purpurin, two natural anthraquinones, have been reported to inhibit tau protein aggregation and have neuroprotective effects [53, 55]. The phenolic hydroxyl groups on the anthraquinone backbone can bind to hydrophobic residues to prevent oligomerization caused by hydrophobic interactions. This feature may explain why anthraquinone derivatives inhibit aggregation more effectively than anthraquinones [56]. The prime interactions leading to the activity of anthraquinone molecules are hydrogen bonds, aromatic contacts, and charge-based contacts [39].

To gain further insights into the molecular interaction of Cassia compounds with Aβ aggregates, in silico studies were undertaken. Molecular docking studies of Cassia compounds with the fibrillar form of α-helical Aβ42 monomer (PDB ID: 1Z0Q), β-sheet Aβ42 monomer, and Aβ42 pentamer (PDB ID: 2BEG) were performed to determine the likely mode of interaction. Test compounds bound to α-helical monomers (1Z0Q) and β-sheet monomers (2BEG) with binding affinities of approximately −4.00 to −5.00 kcal/mol, via hydrophobic, electrostatic, and hydrogen bonding interactions (Tables S1–S3; Figures S1–S3).

A natural flavonol, morin, exhibits a neuroprotective effect by inhibiting AB aggregation via fibril destabilization and the production of off-pathway intermediates [57], inhibition of the formation of IAPP aggregates, and disaggregation of preformed fibrils [58]. In addition, morin binds to monomeric, oligomeric, and fibrillary insulin, thereby preventing conformational changes and avoiding the release of oligomeric species [59]. Therefore, morin is used as a reference natural drug. In the docking study, reference ligand morin and 1Z0Q formed a complex with a binding affinity of −4.66 kcal/mol through three hydrogen-bonding interactions (Gln15, Glu11, Glu22) and a hydrophobic interaction with Glu22 (Table S1 and Figure S1). Morin has three hydrogen bonds (Leu17$^{C,\ E}$, Phe19^{D}) and six hydrophobic residues, Leu17^{E} (π-alkyl), Leu17^{D} (π-σ, π-alkyl), Phe19^{C} (π-π, T form), Val40^{C} (π-σ, π-alkyl), and Val40^{D,E} (π-alkyl), for 2BEG pentamer through binding with a binding affinity of 7.42 kcal/mol (Table S3 and Figure S3). In particular, Cassia anthraquinone and naphthopyrone glycosides bound to α-helix or β-sheet monomers like that of the reference compound morin (Tables S1 and S2; Figures S1 and S2) which also corroborate a recent report [60]. Likewise, with binding affinities of −6.39 to −9.02 kcal/mol via hydrogen bonding, hydrophobic, and π lone pair interactions (Table S3 and Figure S3), these compounds bound to the Aβ42 β-sheet pentamers.

The in vitro anti-glycation assay results show that Cassia compounds inhibit the protein glycation reaction in human insulin. In particular, compounds **5**, **15**, **16**, **20, 24**, and **27** inhibited the glycation of human insulin with IC_{50} values less than 100 μM. Interestingly, compounds **8**, **18**, **24**, **26**, and **33–36** inhibited the glycation of bovine serum albumin with IC_{50} values less than 50 μM, whereas **11** and **38** showed IC_{50} values between 50 and 100 μM. A small change in the functional moiety in the structure greatly affects the bioactivity. Our graphical abstract relates differences in the structures of a series of molecules to differences in their biological activity. However, a detailed structure-activity relationship should be conducted to explore further.

An exposure time of the glycating agent affects the structural changes in the protein. In a study by Khan et al. [61], thymoquinone showed a much more protective effect on the glycation of superoxide dismutase by methylglyoxal at short incubation time (1 h) compared to longer incubation (10 days). In the same study, the band in SDS-PAGE appeared lighter with an increase in the duration of exposure to the sugar, presumably indicating cross-linking and/or degradation into small peptides. In our study, the incubation time is 7 days. So, based on the previous report [61], our test compounds could have a promising anti-glycation effect in a short incubation time.

Proteins exposed to glucose are cleaved and undergo conformational changes, forming fluorescent adducts called "sugar fluorophores." Glycation is a functional and structural change in macromolecules resulting from exposure to high glycemic levels of glucose, caused by the covalent binding of glucose to amino groups of proteins, resulting in surface charges, hydrogen-bonding capacity, cellular recognition, and/or altered formation of cross-linkable complex products [62–64]. Protein fragmentation by

monosaccharides has been observed previously but was not considered equivalent to free radical damage [65]. A study by Hunt et al. demonstrates that monosaccharide incorporation into proteins can be independent of the fragmentation and conformational changes that occur when proteins are exposed to glucose [66]. They also showed that the level of sugar fluorophore formation does not match the extent of protein conformational changes. Thus, the conformational change induced by the exposure of the protein to glucose can be dissociated from the incorporation of the monosaccharide into the protein itself and confirms that free radical and peroxide production must be considered in any situation where biological structures are exposed to high levels of monosaccharides.

Due to the antioxidant properties, several natural compounds are used to treat depression, an example being polyphenolic compounds that modulate neurotransmitter systems and simultaneously induce anti-inflammatory, anti-apoptotic, antigenic toxicity, antimutagenic, and antioxidant effects that can protect against cellular damage [67]. In this study, the ONOO-radical scavenging assay showed that compounds **5**, **8**, **14**, and **33** are strong antioxidants (Table 2). As oxidative stress is directly involved in the pathogenesis of depressive disorders [68], the promising antioxidant effect of Cassia compounds via ONOO-radical scavenging could be neuroprotective, which warrants in vivo studies.

5. Conclusion

In summary, a total of thirty-eight metabolites from Cassia seeds comprising anthraquinones, naphthopyrones, naphthalene, and naphthalenic lactones and their glycosides were tested for anti-glycation, anti-β-amyloid aggregation, and antioxidant effects. Results showed that compounds **9–12, 14–18**, **24**, **25**, **27**, **33–36**, and **38** exhibit good inhibition of AGE formation. Likewise, compounds **5**, **15**, **16**, **20, 24**, and **27** showed good inhibition of D-ribose-mediated glycation of human insulin. In addition, compounds **8** and **12** showed promising inhibition of Aβ aggregation in the thioflavin-T assay, and compounds **5**, **8**, **14**, and **33** scavenged the peroxynitrite anion ($ONOO^-$) at lower concentrations. Molecular docking simulations confirmed that these active compounds have great potential to interact with the Aβ42 peptide and interfere with its self-assembly and conformational transition, and the inhibition of Aβ aggregation prevents full peptide Aβ42 aggregation. Overall, the present study highlights Cassia compounds as potential neuroprotective agents against comorbid AD and diabetes.

Still, in silico molecular dynamics studies predicting the stability of ligand-receptor complexes are lacking. Penetration into the central nervous system and stability of the ligand-receptor complex remain to be studied in vivo. The effects of the tested compounds at the cellular or organismal level remain unknown because in silico modeling cannot account for interactions between compounds and other unrelated targets. In the future, a more detailed understanding of amyloid β-aggregation, especially using in vivo models, will be critical to ensure the activity of these Cassia compounds in comorbid AD and diabetes.

Data Availability

The data supporting the findings of this study are available within the paper and its supplementary files.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

PP and JSC were responsible for conceptualization. PP, SS, and SHS were responsible for methodology. RP, HJL, and SHS were responsible for software. JSC and HAJ were responsible for investigation, validation, and review and editing. PP was responsible for original draft preparation. JSC was responsible for visualization and supervision. All authors have read and approved the final manuscript.

Acknowledgments

This research was supported by a National Research Foundation of Korea (NRF) grant funded by the Ministry of Science and ICT (no. 2020R1C1C1008331 [HNIBR202100303]).

Supplementary Materials

Molecular docking (Figures S1–S3 and Tables S1–S3) of compounds and reference drugs with amyloid-beta peptide. (*Supplementary Materials*)

References

[1] N. Sasaki, R. Fukatsu, K. Tsuzuki et al., "Advanced glycation end products in Alzheimer's disease and other neurodegenerative diseases," *American Journal Of Pathology*, vol. 153, no. 4, pp. 1149–1155, 1998.

[2] S. Yan, X. Chen, A. Schmidt et al., "Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress," *Proceedings of the National Academy of Sciences*, vol. 91, no. 16, pp. 7787–7791, 1994.

[3] M. P. Vitek, K. Bhattacharya, J. M. Glendening et al., "Advanced glycation end products contribute to amyloidosis in Alzheimer disease," *Proceedings of the National Academy of Sciences*, vol. 91, no. 11, pp. 4766–4770, 1994.

[4] Y. H. Abdel-Wahab, F. P. O'Harte, H. Ratcliff, N. H. McClenaghan, C. R. Barnett, and P. R. Flatt, "Glycation of insulin in the islets of Langerhans of normal and diabetic animals," *Diabetes*, vol. 45, no. 11, pp. 1489–1496, 1996.

[5] L. Breydo, J. W. Wu, and V. N. Uversky, "α-Synuclein misfolding and Parkinson's disease," *Biochimica et Biophysica Acta- Molecular Basis of Disease*, vol. 1822, no. 2, pp. 261–285, 2012.

[6] B. Bouma, L. M. Kroon-Batenburg, Y.-P. Wu et al., "Glycation induces formation of amyloid cross-β structure in albumin," *Journal of Biological Chemistry*, vol. 278, no. 43, pp. 41810–41819, 2003.

[7] D. Tagliazucchi, S. Martini, and A. Conte, "Protocatechuic and 3,4-dihydroxyphenylacetic acids inhibit protein glycation by binding lysine through a metal-catalyzed oxidative mechanism," *Journal of Agricultural and Food Chemistry*, vol. 67, no. 28, pp. 7821–7831, 2019.

[8] M. Snelson and M. T. Coughlan, "Dietary advanced glycation end products: digestion, metabolism and modulation of gut microbial ecology," *Nutrients*, vol. 11, no. 2, p. 215, 2019.

[9] H. Younus and S. Anwar, "Prevention of non-enzymatic glycosylation (glycation): implication in the treatment of diabetic complication," *International Journal of Health Sciences*, vol. 10, no. 2, pp. 247–263, 2016.

[10] C. Shantharam, D. Suyoga Vardhan, R. Suhas, M. Sridhara, and D. C. Gowda, "Inhibition of protein glycation by urea and thiourea derivatives of glycine/proline conjugated benzisoxazole analogue–Synthesis and structure–activity studies," *European Journal of Medicinal Chemistry*, vol. 60, pp. 325–332, 2013.

[11] K. M. Khan, M. Khan, M. Ali et al., "Synthesis of bis-Schiff bases of isatins and their antiglycation activity," *Bioorganic & Medicinal Chemistry*, vol. 17, no. 22, pp. 7795–7801, 2009.

[12] M. Taha, A. Mosaddik, F. Rahim, S. Ali, M. Ibrahim, and N. B. Almandil, "Synthesis, antiglycation and antioxidant potentials of benzimidazole derivatives," *Journal of King Saud University Science*, vol. 32, no. 1, pp. 191–194, 2020.

[13] K. M. Khan, M. Khan, N. Ambreen et al., "Oxindole derivatives: synthesis and antiglycation activity," *Medicinal Chemistry*, vol. 9, no. 5, pp. 681–688, 2013.

[14] T. Vijaya Kumar, A. K. Tiwari, A. Robinson et al., "Synthesis and antiglycation potentials of bergenin derivatives," *Bioorganic & Medicinal Chemistry Letters*, vol. 21, no. 16, pp. 4928–4931, 2011.

[15] S. Ullah, M. Saeed, I. Ullah et al., "Synthesis and characterization of novel piroxicam derivatives and their antiglycation activity," *Journal of Molecular Structure*, vol. 1239, Article ID 130470, 2021.

[16] I. J. Cho, C. Lee, and T. Y. Ha, "Hypolipidemic effect of soluble fiber isolated from seeds of *Cassia tora* Linn. in rats fed a high-cholesterol diet," *Journal of Agricultural and Food Chemistry*, vol. 55, no. 4, pp. 1592–1596, 2007.

[17] T.-F. Tzeng, H.-J. Lu, S.-S. Liou, C. J. Chang, and I.-M. Liu, "Reduction of lipid accumulation in white adipose tissues by *Cassia tora* (Leguminosae) seed extract is associated with AMPK activation," *Food Chemistry*, vol. 136, no. 2, pp. 1086–1094, 2013.

[18] Y.-M. Kim, C.-H. Lee, H.-G. Kim, and H.-S. Lee, "Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi," *Journal of Agricultural and Food Chemistry*, vol. 52, no. 20, pp. 6096–6100, 2004.

[19] S. H. Chan, A. Koo, and K. Li, "The involvement of medullary reticular formation in the hypotensive effect of extracts from seeds of *Cassia tora*," *The American Journal of Chinese Medicine*, vol. 4, no. 4, pp. 383–389, 1976.

[20] G.-C. Yen and D.-Y. Chung, "Antioxidant effects of extracts from *Cassia tora* L. prepared under different degrees of roasting on the oxidative damage to biomolecules," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 4, pp. 1326–1332, 1999.

[21] B. D. Drever, W. G. Anderson, G. Riedel et al., "The seed extract of *Cassia obtusifolia* offers neuroprotection to mouse hippocampal cultures," *Journal of Pharmacological Sciences*, vol. 107, no. 4, pp. 380–392, 2008.

[22] Y.-C. Yang, M.-Y. Lim, and H.-S. Lee, "Emodin isolated from *Cassia obtusifolia* (Leguminosae) seed shows larvicidal activity against three mosquito species," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 26, pp. 7629–7631, 2003.

[23] J. S. Choi, H. J. Lee, and S. S. Kang, "Alatemin, cassiaside and rubrofusarin gentiobioside, radical scavenging principles from the seeds of *Cassia tora* on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical," *Archives of Pharmacal Research*, vol. 17, no. 6, pp. 462–466, 1994.

[24] H. A. Jung, M. Y. Ali, H. J. Jung, H. O. Jeong, H. Y. Chung, and J. S. Choi, "Inhibitory activities of major anthraquinones and other constituents from *Cassia obtusifolia* against β-secretase and cholinesterases," *Journal of Ethnopharmacology*, vol. 191, pp. 152–160, 2016.

[25] P. Paudel, H. A. Jung, and J. S. Choi, "Anthraquinone and naphthopyrone glycosides from *Cassia obtusifolia* seeds mediate hepatoprotection via Nrf2-mediated HO-1 activation and MAPK modulation," *Archives of Pharmacal Research*, vol. 41, no. 6, pp. 677–689, 2018.

[26] H. A. Jung, M. D. Ali, and J. S. Choi, "Promising inhibitory effects of anthraquinones, naphthopyrone, and naphthalene glycosides, from *Cassia obtusifolia* on α-glucosidase and human protein tyrosine phosphatases 1B," *Molecules*, vol. 22, no. 1, p. 28, 2016.

[27] S. K. Hyun, H. Lee, S. S. Kang, H. Y. Chung, and J. S. Choi, "Inhibitory activities of *Cassia tora* and its anthraquinone constituents on angiotensin-converting enzyme," *Phytotherapy Research*, vol. 23, no. 2, pp. 178–184, 2009.

[28] P. Paudel, S. H. Seong, H. A. Jung, and J. S. Choi, "Rubrofusarin as a dual protein tyrosine phosphate 1B and human monoamine oxidase-A inhibitor: an in vitro and in silico study," *ACS Omega*, vol. 4, no. 7, pp. 11621–11630, 2019.

[29] J. S. Choi, H. J. Lee, K.-Y. Park, J.-O. Ha, and S. S. Kang, "In vitro antimutagenic effects of anthraquinone aglycones and naphthopyrone glycosides from *Cassia tora*," *Planta Medica*, vol. 63, no. 1, pp. 11–14, 1997.

[30] G. Y. Lee, D. S. Jang, Y. M. Lee, J. M. Kim, and J. S. Kim, "Naphthopyrone glucosides from the seeds of *Cassia tora* with inhibitory activity on advanced glycation end products (AGEs) formation," *Archives of Pharmacal Research*, vol. 29, no. 7, pp. 587–590, 2006.

[31] D. S. Jang, G. Y. Lee, Y. S. Kim et al., "Anthraquinones from the seeds of *Cassia tora* with inhibitory activity on protein glycation and aldose reductase," *Biological and Pharmaceutical Bulletin*, vol. 30, no. 11, pp. 2207–2210, 2007.

[32] P. Paudel, S. H. Seong, S. Shrestha, H. A. Jung, and J. S. Choi, "In vitro and in silico human monoamine oxidase inhibitory potential of anthraquinones, naphthopyrones, and naphthalenic lactones from *Cassia obtusifolia* Linn seeds," *ACS Omega*, vol. 4, no. 14, pp. 16139–16152, 2019.

[33] J. A. Vinson and T. B. Howard III, "Inhibition of protein glycation and advanced glycation end products by ascorbic acid and other vitamins and nutrients," *The Journal of Nutritional Biochemistry*, vol. 7, no. 12, pp. 659–663, 1996.

[34] C. Iannuzzi, M. Borriello, A. D'Agostino, D. Cimini, C. Schiraldi, and I. Sirangelo, "Protective effect of extractive and biotechnological chondroitin in insulin amyloid and advanced glycation end product-induced toxicity," *Journal of Cellular Physiology*, vol. 234, no. 4, pp. 3814–3828, 2019.

[35] L. S. Wolfe, M. F. Calabrese, A. Nath, D. V. Blaho, A. D. Miranker, and Y. Xiong, "Protein-induced photophysical changes to the amyloid indicator dye thioflavin T," *Proceedings of the National Academy of Sciences*, vol. 107, no. 39, pp. 16863–16868, 2010.

[36] S. Kim, Y. Nam, S. J. Shin et al., "Dual modulators of aggregation and dissociation of amyloid beta and tau: in vitro, in vivo, and in silico studies of Uncaria rhynchophylla and its bioactive components," *Biomedicine & Pharmacotherapy*, vol. 156, Article ID 113865, 2022.

[37] N. W. Kooy, J. A. Royall, H. Ischiropoulos, and J. S. Beckman, "Peroxynitrite-mediated oxidation of dihydrorhodamine 123," *Free Radical Biology and Medicine*, vol. 16, no. 2, pp. 149–156, 1994.

[38] E. Cho, M. Cai, H. Kwon et al., "Rubrofusarin inhibits Aβ aggregation and ameliorates memory loss in an Aβ-induced Alzheimer's disease-like mouse model," *Food and Chemical Toxicology*, vol. 132, Article ID 110698, 2019.

[39] M. Convertino, R. Pellarin, M. Catto, A. Carotti, and A. Caflisch, "9, 10-Anthraquinone hinders β-aggregation: how does a small molecule interfere with Aβ-peptide amyloid fibrillation?" *Protein Science*, vol. 18, no. 4, pp. 792–800, 2009.

[40] M. Hirohata, K. Hasegawa, S. Tsutsumi-Yasuhara et al., "The anti-amyloidogenic effect is exerted against Alzheimer's β-amyloid fibrils in vitro by preferential and reversible binding of flavonoids to the amyloid fibril structure," *Biochemistry*, vol. 46, no. 7, pp. 1888–1899, 2007.

[41] M. Brownlee, "The pathological implications of protein glycation," *Clinical and investigative medicine. Medecine clinique et experimentale*, vol. 18, no. 4, pp. 275–281, 1995.

[42] Y. H. A. Abdel-Wahab, F. P. M. O'Harte, A. C. Boyd, C. R. Barnett, and P. R. Flatt, "Glycation of insulin results in reduced biological activity in mice," *Acta Diabetologica*, vol. 34, no. 4, pp. 265–270, 1997.

[43] C. Iannuzzi, M. Borriello, V. Carafa et al., "D-ribose-glycation of insulin prevents amyloid aggregation and produces cytotoxic adducts," *Biochimica et Biophysica Acta- Molecular Basis of Disease*, vol. 1862, no. 1, pp. 93–104, 2016.

[44] S. Takeda, N. Sato, H. Rakugi, and R. Morishita, "Molecular mechanisms linking diabetes mellitus and Alzheimer disease: beta-amyloid peptide, insulin signaling, and neuronal function," *Molecular BioSystems*, vol. 7, no. 6, pp. 1822–1827, 2011.

[45] A. Ahmad, V. N. Uversky, D. Hong, and A. L. Fink, "Early events in the fibrillation of monomeric insulin," *Journal of Biological Chemistry*, vol. 280, no. 52, pp. 42669–42675, 2005.

[46] D.-P. Hong, A. Ahmad, and A. L. Fink, "Fibrillation of human insulin A and B chains," *Biochemistry*, vol. 45, no. 30, pp. 9342–9353, 2006.

[47] V. P. Singh, A. Bali, N. Singh, and A. S. Jaggi, "Advanced glycation end products and diabetic complications," *KOREAN JOURNAL OF PHYSIOLOGY and PHARMACOLOGY*, vol. 18, no. 1, p. 1, 2014.

[48] P. Alavi, R. Yousefi, S. Amirghofran, H. R. Karbalaei-Heidari, and A. A. Moosavi-Movahedi, "Structural analysis and aggregation propensity of reduced and nonreduced glycated insulin adducts," *Applied Biochemistry and Biotechnology*, vol. 170, no. 3, pp. 623–638, 2013.

[49] L. Oliveira, A. Lages, R. A. Gomes et al., "Insulin glycation by methylglyoxal results in native-like aggregation and inhibition of fibril formation," *BMC Biochemistry*, vol. 12, no. 1, pp. 41–13, 2011.

[50] N. Apetz, G. Munch, S. Govindaraghavan, and E. Gyengesi, "Natural compounds and plant extracts as therapeutics against chronic inflammation in Alzheimer's disease–a translational perspective," *CNS & Neurological Disorders-Drug Targets*, vol. 13, no. 7, pp. 1175–1191, 2014.

[51] H. Jahan and M. I. Choudhary, "Glycation, carbonyl stress and AGEs inhibitors: a patent review," *Expert Opinion on Therapeutic Patents*, vol. 25, no. 11, pp. 1267–1284, 2015.

[52] S. Anwar, M. A. Khan, A. Sadaf, and H. Younus, "A structural study on the protection of glycation of superoxide dismutase by thymoquinone," *International Journal of Biological Macromolecules*, vol. 69, pp. 476–481, 2014.

[53] M. Pickhardt, Z. Gazova, M. von Bergen et al., "Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments in vitro and in cells," *Journal of Biological Chemistry*, vol. 280, no. 5, pp. 3628–3635, 2005.

[54] M. Abu-Hussien, G. K. Viswanathan, L. Borisover et al., "Inhibition of amyloid fibrillation of γD-crystallin model peptide by the cochineal Carmine," *International Journal of Biological Macromolecules*, vol. 169, pp. 342–351, 2021.

[55] G. K. Viswanathan, D. Shwartz, Y. Losev et al., "Purpurin modulates Tau-derived VQIVYK fibrillization and ameliorates Alzheimer's disease-like symptoms in animal model," *Cellular and Molecular Life Sciences*, vol. 77, no. 14, pp. 2795–2813, 2020.

[56] H. Gong, Z. He, A. Peng et al., "Effects of several quinones on insulin aggregation," *Scientific Reports*, vol. 4, no. 1, p. 5648, 2014.

[57] J. A. Lemkul and D. R. Bevan, "Morin inhibits the early stages of amyloid β-peptide aggregation by altering tertiary and quaternary interactions to produce "off-pathway" structures," *Biochemistry*, vol. 51, no. 30, pp. 5990–6009, 2012.

[58] H. Noor, P. Cao, and D. P. Raleigh, "Morin hydrate inhibits amyloid formation by islet amyloid polypeptide and disaggregates amyloid fibers," *Protein Science*, vol. 21, no. 3, pp. 373–382, 2012.

[59] P. Patel, K. Parmar, and M. Das, "Inhibition of insulin amyloid fibrillation by Morin hydrate," *International Journal of Biological Macromolecules*, vol. 108, pp. 225–239, 2018.

[60] A. Radwan and F. Alanazi, "Combined modeling study of the binding characteristics of natural compounds, derived from Psoralea fruits, to β-amyloid peptide monomer," *International Journal of Molecular Sciences*, vol. 23, no. 7, p. 3546, 2022.

[61] M. A. Khan, S. Anwar, A. N. Aljarbou et al., "Protective effect of thymoquinone on glucose or methylglyoxal-induced glycation of superoxide dismutase," *International Journal of Biological Macromolecules*, vol. 65, pp. 16–20, 2014.

[62] A. Cerami, "Aging of proteins and nucleic acids: what is the role of glucose?" *Trends in Biochemical Sciences*, vol. 11, no. 8, pp. 311–314, 1986.

[63] S. Pongor, P. C. Ulrich, F. A. Bencsath, and A. Cerami, "Aging of proteins: isolation and identification of a fluorescent chromophore from the reaction of polypeptides with glucose," *Proceedings of the National Academy of Sciences*, vol. 81, no. 9, pp. 2684–2688, 1984.

[64] J. J. Harding, "Nonenzymatic covalent posttranslational modification of proteins in vivo," *Advances in Protein Chemistry*, vol. 37, pp. 247–334, 1985.

[65] H. T. Beswick and J. J. Harding, "Conformational changes induced in lens alpha- and gamma-crystallins by modification with glucose 6-phosphate. Implications for cataract," *Biochemical Journal*, vol. 246, no. 3, pp. 761–769, 1987.

[66] J. V. Hunt, R. T. Dean, and S. P. Wolff, "Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental

glycation model of diabetes mellitus and ageing," *Biochemical Journal*, vol. 256, no. 1, pp. 205–212, 1988.

[67] N. Cervantes-Anaya, G. Azpilcueta-Morales, E. Estrada-Camarena et al., "Pomegranate and its components, punicalagin and ellagic acid, promote antidepressant, antioxidant, and free radical-scavenging activity in ovariectomized rats," *Frontiers in Behavioral Neuroscience*, vol. 16, Article ID 836681, 2022.

[68] M. Maes, P. Galecki, Y. S. Chang, and M. Berk, "A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 35, no. 3, pp. 676–692, 2011.