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"The development of anti-glycating Lonicera japonica plant extract through molecular docking and virtual screening of 13 plant-derived phenolic compounds for anti-glycation targets of aldose reductase, RAGE and PPARG."

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0. Abstract

The covalent adducts generated by the glycation are referred to as advanced glycation end products (AGEs). Receptor for AGEs (RAGE) mediates effects of AGEs and is associated with increased oxidative stress, cell growth and inflammation. 13 plant compounds with predictive anti-glycation effect were listed by literature survey. The candidates of effective anti-glycation compounds were predicted by molecular docking simulation for anti-glycation targets of aldose reductase (AR), RAGE and peroxisome proliferator-activated receptor gamma (PPARG). 3 plants of Lonicera japonica, Stevia rebaudiana and Ilex cornuta with predictive anti-glycation compounds were selected by literature survey. These 3 plants were extracted and analyzed by HPLC-MS. The experiments for anti-glycation effects were performed on 3 whole plant extracts. In vitro assessments using human skin fibroblast (HSF) models demonstrated that all three extracts exhibited significant anti-glycation effects through multiple mechanisms such as suppression of AGEs formation, inhibition of collagen cross-linking, prevention of vimentin aggregation, and downregulation of both AGEs accumulation and RAGE expression. Notably, Lonicera japonica Thunb. extract displayed superior efficacy across all evaluated parameters. These findings suggest promising applications for plant-derived anti-glycation agents in cosmetic formulations targeting skin glycation processes.

Keywords: Aging signs evaluation, AI, Algorithms, Anti-aging, Ingredients combination

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1. Introduction

Non-enzymatic glycation (NEG), commonly known as the glycation reaction, refers to the non-enzymatic condensation reaction that occurs between the carbonyl group of reducing sugars and the free amino groups on macromolecules like proteins, lipids, or nucleic acids[1,2]. The stable covalent adducts generated by the NEG reaction are collectively referred to as advanced glycation end products (AGEs). Human skin contains abundant collagen and elastin, predominantly existing in fibrous forms that constitute the dermal skeletal structure, endowing the skin with mechanical strength and elasticity. AGEs induce protein cross-linking through intermolecular interactions, altering the physical properties of collagen and elastin. These modifications include increased stiffness, changes in breaking load, denaturation temperature, and solubility[3]. AGEs not only disrupt their function as the optimal scaffold supporting the dermis but also affect skin cell adhesion and cell growth[4,5].

Various cells exposed to AGEs, such as fibroblasts, will exhibit obvious apoptosis phenomena[6]. At the same time, the accumulation of AGE-protein cross-linkages in the extracellular matrix not only reduces the permeability of connective tissues but also weakens the diffusion ability of nutrients and metabolic waste in the body. The increased hardness of the skin tissue leads to a decrease in skin elasticity and ultimately results in skin aging. In addition to directly inducing changes in the structure of intracellular proteins, AGEs can also trigger a series of diseases by binding to cell receptors. The receptor for advanced glycation end products (RAGE) is the most common specific receptor for AGEs[7,8]. When AGEs bind to RAGE, some intracellular signaling pathways will be activated, including the nicotinamide adenine dinucleotide phosphate oxidase, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) signaling pathways. These pathways will induce the expression of transcription factors related to stress and inflammation, such as activator protein-1 (AP-1), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and signal transducer and activator of transcription 3 (STAT3), and further regulate the gene expression of monocyte chemoattractant protein-1, vascular endothelial growth factor, vascular cell adhesion molecule, endothelin-1, E-selectin, matrix metalloproteinases, and inflammatory cytokines. Eventually, this leads to the occurrence of inflammation, oxidative stress, and even aging, chronic diseases, and cancer[9,10].

Anti-glycation mechanisms primarily involve three strategies: inhibition of AGE formation, acceleration of AGE or AGE crosslink catabolism, and blockade of AGE biological effects[11,12]. Aldose reductase is a key enzyme in the polyol pathway. In the polyol pathway, glucose is converted into fructose, and fructose is more easily converted into α-dicarbonyl compounds or AGEs. Therefore, inhibiting the polyol pathway is also a potential way to reduce the formation of AGEs[13]. Since AGEs easily bind to RAGE and trigger various diseases, blocking the binding and expression of AGEs and RAGE is also an effective approach[14]. This can be achieved by activating the activity of peroxisome proliferator-activated receptor γ to inhibit the expression of RAGE or directly binding to RAGE to block its interaction with ligands[15-17]. Recent research results have shown that chlorogenic acid has biological activities such as antioxidant, anti-inflammatory, antibacterial, and antiviral properties. Results from population epidemiological surveys, intervention studies, and animal experiments have demonstrated that chlorogenic acid plays a positive role in regulating glucose and lipid metabolism, improving insulin resistance, and reducing the risks of type 2 diabetes and cardiovascular diseases[18]. The aim of this study is to explore the roles and mechanisms of plant extracts rich in different types of chlorogenic acid in eliminating the effects of AGEs.

The results will contribute to supporting the application of chlorogenic acid and plant extracts in the field related to skin anti-glycation.

2. Materials and Methods

Molecular docking Firstly, the 2D structure of the ligands in SDF format was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov), which was transformed into the 3D structure using the Chem3D software, and the structures were optimized according to the minimum energy to obtain the most stable molecular structure. Then, the receptor structures in PDB format were obtained from the Protein Sequence Database (https://www.rcsb.org), and excess chains, ions, and water molecules were eliminated by the PyMOL software. Afterward, the AutoDockTools software was used to add hydrogen atoms in receptors and convert the format of ligands and receptors. Finally, the molecular docking was performed by the AutoDock Vina software[19], the PyMOL software was used to visualize the molecular docking results.

Plant extraction and phytochemical analysis Plant material (50 g) was homogenized and subjected to ultrasonic-assisted extraction [70% ethanol, solid-to-liquid ratio of 1:20 (w/v)] at 40°C for 90 min. The supernatant was filtered, concentrated via rotary evaporation, and lyophilized to obtain crude extract. The contents of different types of chlorogenic acids in the plant extract are detected by high-performance liquid chromatography-mass spectrometry (HPLC-MS).

AGEs formation inhibition assay A glycation model was established by incubating 0.5 M glucose with 10 mg/mL bovine serum albumin (BSA) in 0.1 M phosphate-buffered saline (PBS, pH 7.4, 0.02% sodium azide) at 37°C for 21 days. Fluorescence intensity (excitation 370 nm, emission 440 nm) was measured using a microplate reader. Aminoguanidine served as the positive control. Inhibition rates were calculated as:

$$\text{Inhibition}(\%) = \left(1 - \frac{F_{sample}}{F_{control}}\right) \times 100$$

Collagen cross-linking suppression assay Human dermal fibroblasts (HDFs, passage 3-5) were seeded in 24-well plates and cultured with 400 μM Glyoxal and plant extracts for 5 days. The cells were recovered with Trypsin, resuspended with Fetal bovine serum (FBS), mixed into the collagen mixture, and cultured on a 24-well plate treated with Anti-Link™ (Allvivo Vascular) for 1 hour. Then, a medium containing 10% FBS was added to the upper layer. After culturing for 24 hours, the number of collagen Cross-linking was determined.

Cytoskeletal vimentin aggregation assay HDFs treated with glyoxal and plant extracts were fixed with 3.7% paraformaldehyde, permeabilized (0.1% Triton X-100), and incubated with anti-vimentin primary antibody (1:200) followed by Alexa Fluor 488-conjugated secondary antibody (1:500). Cytoskeletal morphology was visualized using confocal laser scanning microscopy (Leica TCS SP8), with filament integrity quantified via Image J software.

AGEs and RAGE expression analysis. Post-treatment cells were lysed in RIPA buffer. supplemented with protease inhibitors. Protein concentrations were determined via BCA assay. Equal amount of protein were separated by 10% SDS-PAGE and transferred to PVDF membranes. The expression level of AGEs and RAGE was determined by Immunoblot.

Statistical analyses All values are mean ±SEM of at least 3 separated experiments. Comparisons between groups were analyzed using t-test. The p-values <0.05 were considered statistically significant.

3. Results

Molecular docking study To evaluate the binding affinities of 13 candidate compounds to anti-glycation therapeutic targets, molecular docking simulations were performed using AutoDock Vina against the crystallographic structures of Aldose Reductase (AR, PDB: 1el3), RAGE (PDB: 6xq1), and PPARγ (PDB: 2g0g).

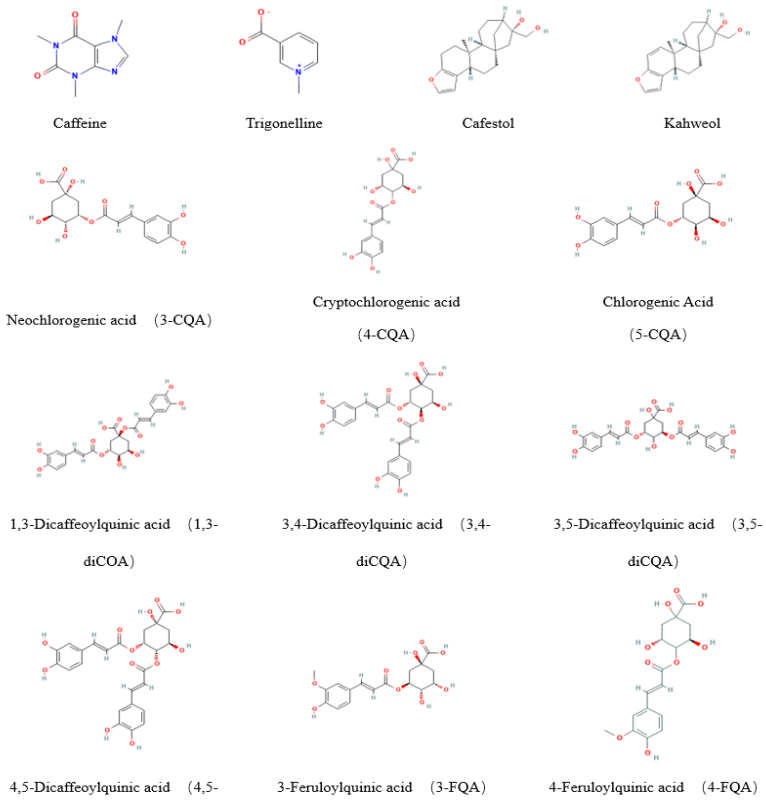

Figure 1. The chemical structures of the 13 compounds

Table 1. Binding energies of 13 molecules to 3 anti-glycation targets

Compounds	Aldose reductase	RAGE	PPARG
Caffeine	-6	-4.7	-5.8
Trigonelline	-5.3	-4.6	-5.5
Cafestol	-8.9	-6.4	-8.7
Kahweol	-9	-6.4	-8.7
3-CQA	-8	-6.7	-8.3

4-CQA	-8.3	-6.6	-8.4
5-CQA	-8.3	-7	-8
1,3-diCOA	-8.9	-6.2	-9.7
3,4-diCQA	-8.6	-7.8	-9.8
3,5-diCQA	-9.5	-7.2	-10.3
4,5-diCQA	-9.2	-7.2	-9.1
3-FQA	-8.4	-6.6	-8.4
4-FQA	-8.4	-6.7	-8.5

Figure 1 displays the chemical structures of the 13 compounds. Binding energies (ΔG, kcal/mol) were quantified to assess receptor-ligand complex stability, where lower values indicate stronger interactions and higher pharmacological potential[20]. Table 1 presents the docking binding energies of 13 compounds with 3 anti-glycation-related targets. Phenolic compounds exhibited distinct binding energies across the targets: AR interactions demonstrated docking energies ranging from -9.5 to -8.0 kcal/mol, RAGE interactions spanned -7.8 to -6.2 kcal/mol, and PPARG binding energies ranged from -10.3 to -8.0 kcal/mol.

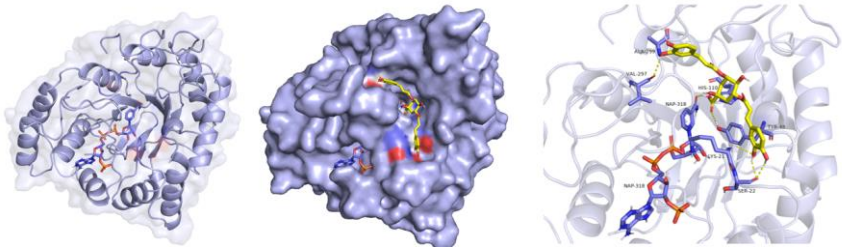

Figure 2. The 3D interactions of 3,5-diCQA and AR.

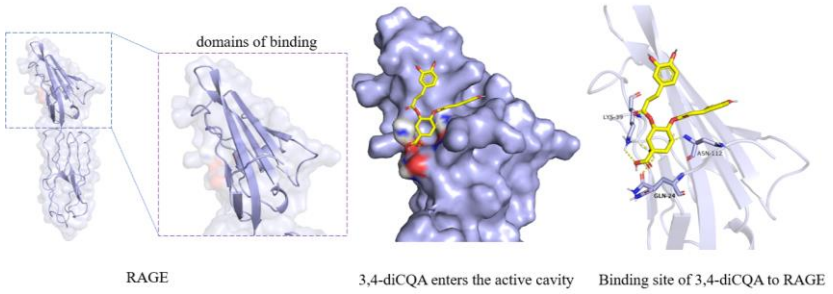

Figure 3. The 3D interactions of 3,4-diCQA and RAGE.

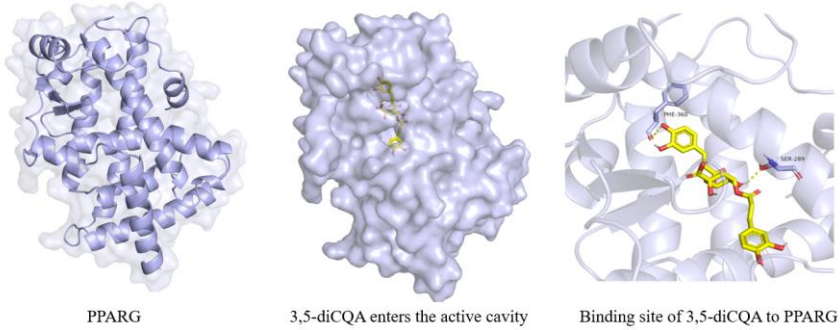

Figure 4. The 3D interactions of 3,5-diCQA and PPARG.

Structural analysis of the most stable complexes identified critical hydrogen-bonding interactions. As shown in Figure 2, 3,5-diCQA formed hydrogen bonds with VAL-297, ALA-299, HIS-110, TYR-48, LYS-21, and SER-22 residues in AR. Figure 3 illustrates 3,4-diCQA interacting with LYS-39, ASN-112, and GLN-24 residues in RAGE, while Figure 4 demonstrates 3,5-diCQA binding to SER-289 and PHE-360 residues in PPARG through hydrogen bonds. These interactions suggest molecular mechanisms underlying the observed anti-glycation potential.

Table 2. The target compound content (μg/g) in 3 plant extracts

Compound	*L. japonica*	*S. rebaudiana*	*I. cornuta*
3,4-diCQA	250.2	200.5	N.D.
3,5-diCQA	934.3	779.7	418.7
4,5-diCQA	118.2	206.2	98.75

Phytochemical constituents in plant extracts Three plant extracts containing characterized chlorogenic acid (CQA) derivatives with anti-glycation potential were prepared via ultrasonic-assisted low temperature extraction and analyzed by HPLC-MS. As shown in Table 2, the Lonicera japonica Thunb. extract exhibited the highest total content of 3,4-diCQA, 3,5-diCQA, and 4,5-diCQA isomers (250.2, 934.3, and 118.2 μg/g, respectively), followed by *Stevia rebaudiana* and *Ilex cornuta* extracts (N.D. indicates "not detected").

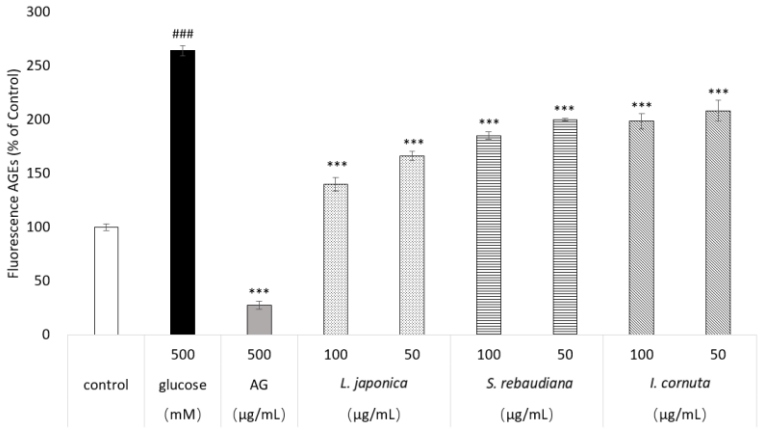

Figure 5. Inhibition effects of AGEs formation

Inhibitory effects on AGEs formation Figure 5 shows the inhibitory effect of plant extracts on AGEs formation. In vitro experiments revealed that 500 mM glucose and BSA co-incubation induced a significant increase in fluorescent advanced glycation end products (AGEs). Aminoguanidine, as a positive control, significantly reduced the generation of fluorescent AGEs. All three plant extracts significantly decreased the generation of fluorescent AGEs ($p<0.001$ vs. model group) and exhibited a dose-dependent relationship. Among them, *L. japonica* extract demonstrating the strongest inhibition.

Modulation of collagen cross-linking in HDF cells Collagen is involved in the non-enzymatic glycation reaction to form advanced glycation end products (AGEs), resulting in the formation of cross-

linked products of collagen. As the cross-linking of collagen gradually intensifies, its solubility decreases, and the permeability of the skin tissue reduces. This leads to obstacles in the exchange of nutrients and metabolites between cells. Eventually, it gives rise to a decrease in skin elasticity, skin relaxation, and the appearance of wrinkles[21].

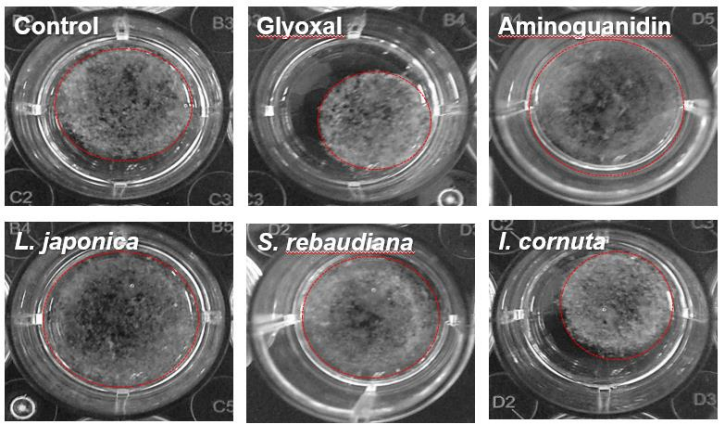

Figure 6. Collagen cross-links in HDF cells

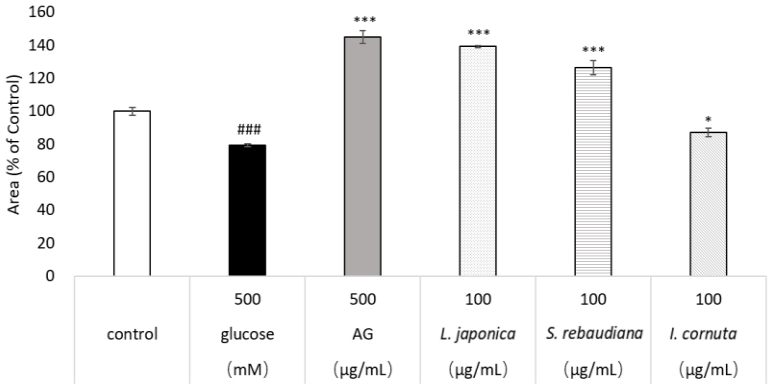

Figure 7. The area statistics of collagen cross-linking.

Figure 6 shows the results of collagen cross-linking in HDF cells, and Figure 7 shows the area statistics of collagen cross-linking. Under the action of glyoxal, the degree of collagen cross-linking deepens. Aminoguanidine, as a positive control, significantly reduces the binding of collagen. Both the plant extracts of L. japonica and S. rebaudiana extremely significantly reduce the degree of collagen cross-linking ($p < 0.001$). Thereby inhibiting the increase in the hardness and the decrease in the elasticity of the extracellular matrix.

Suppression of cytoskeletal glycation Vimentin, as an intermediate filament, forms the cytoskeletal framework together with microtubules and actin microfilaments, participating in numerous critical cellular biological functions. Vimentin serves as the primary target for glycation modification in fibroblasts, with carboxymethyllysine (CML)-modified vimentin polymers predominantly localized in the perinuclear region. The formation of vimentin polymers significantly impairs fibroblast contractile capacity[22].

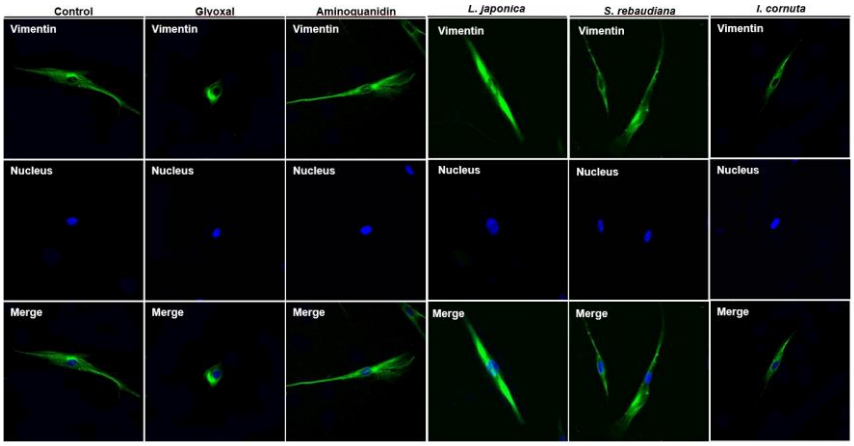

Figure 8. Cytoskeletal glycation in HDF cells

Figure 8 shows immunofluorescence localization of vimentin in HDF cells, and aggregation of vimentin fibers induced by glycosylation can be observed. Under aminoguanidine treatment, vimentin maintained an extended conformational state. All three plant extracts effectively inhibited vimentin filament aggregation, thereby better maintaining the cytoskeleton. Notably, *L. japonica* and *S. rebaudiana* extracts exhibited superior anti-glycation activity to *I. cornuta*, consistent with their higher 3,4- and 3, 5-diCQA content.

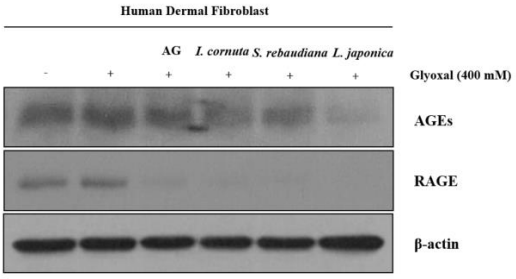

Figure 9. AGEs and RAGE expression in HDF cells

Downregulation of AGEs-RAGE axis AGEs are stable products generated from non-enzymatic reactions between sugars and biological macromolecules such as proteins. In the positive control group (with only glyoxal added), the expression level of AGEs was relatively high. When compared with the positive control group, the expression levels of AGEs in the groups treated with plant extracts (*I. cornuta, S. rebaudiana, Lonicera japonica*) and the aminoguanidine - treated group were all decreased. This indicates that both aminoguanidine and the three plant extracts may inhibit the generation of AGEs induced by glyoxal. Notably, the group treated with Lonicera japonica extract showed a relatively more significant decrease in AGEs expression, suggesting a relatively better inhibitory effect. RAGE is a receptor on the cell surface that recognizes and binds to AGEs. In the positive control group, RAGE was expressed to a certain extent. After treatment with aminoguanidine and the respective plant extracts, the expression level of RAGE decreased significantly. In particular, in the group treated with *L. japonica* extract, RAGE was almost undetectable. This demonstrates that the three plant extracts can not only inhibit the generation of AGEs, but also reduce the expression of RAGE.

4. Discussion

3,5-diCQA, 3,4-diCQA and 4,5-diCQA among 13 phenolic compounds have relatively strong binding affinities of approximately -7.8 ~ -9.5 kcal/mol for aldose reductase, RAGE and PPARG. Among selected 3 plants, L. japonica plant extract had the highest content of 3,5-diCQA and 3,4-diCQA, respectively. In vitro assessments using human skin fibroblast (HSF) models demonstrated that L. japonica extract among three plant extracts exhibited the highest anti-glycation effects through multiple mechanisms such as suppression of AGEs formation (figure 5), inhibition of collagen cross-linking, prevention of vimentin aggregation (Figure 6) and inhibition of AGEs accumulation as the same time as the low level of RAGE expression (Figure 9). Therefore, L. japonica plant extract are predicted to be more effective in anti-glycation. The L. japonica extract could be reduced the generation and action of AGEs by binding to glycation-related targets. Although in vitro assessments of each single compound have not been conducted, it is expected that 3 compounds (3,5-diCQA, 3,4-diCQA and 4,5-diCQA) of L. japonica extract had a great influence on the inhibition of skin glycation and signal transduction by the generated AGEs. From these results, the excellent anti-glycating effect of L. japonica extract are presumed to be due to the effects of 3,5-diCQA, 3,4-diCQA and 4,5-diCQA.

5. Conclusion

3,5-diCQA show the strongest affinity of -9.5 and -10.3 for aldose reductase and PPARG, respectively. 1,3-diCOA, 3,4-diCQA, and 4,5-diCQA show the strong affinities of -8.9, -8.6, -9.2 and -9.7, -9.8, -9.1 for aldose reductase and PPARG, respectively. Therefore, 1,3-diCOA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA could be agonist of PPARG. And also, 3,5-diCQA and 4,5-diCQA could be inhibitor of Aldose reductase. However, 4 compounds of 1,3-diCOA, 3,4-diCQA, 3,5-diCQA and 4,5-diCQA have relatively not strong affinity for RAGE and so could not be inhibitor of RAGE. *L. japonica* extract exhibited potent anti-glycation activity, driven by its high 3,5-diCQA content. This isomer showed strong binding (-9.5 to -10.3 kcal/mol) to key targets (AR/RAGE/PPARγ) via H-bonds. The extract inhibited AGEs formation, reduced collagen cross-linking, and suppressed AGEs-RAGE axis activation in HDF cells, demonstrating dual anti-glycation mechanisms linked to its diCQA profile.

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