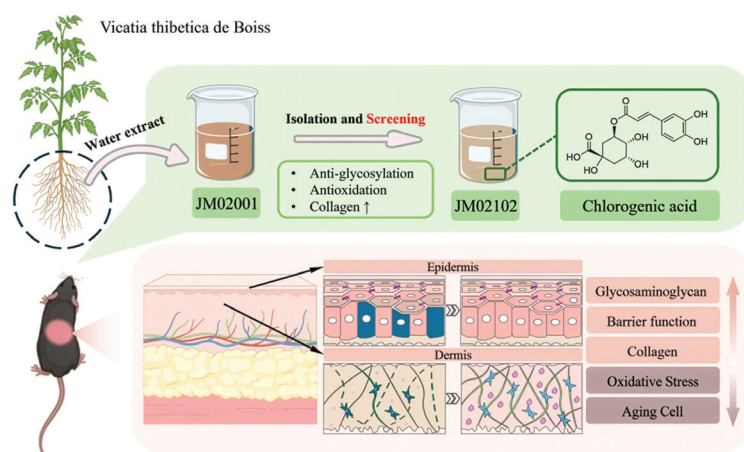


Vicatia thibetica de Boiss root extract and its active constituents attenuate skin aging induced by D-galactose and glucocorticoid

Graphical abstract



Highlights

- *Vicatia thibetica* de Boiss root extract (JM02001) mitigates skin aging *in vivo* by preventing extracellular matrix (ECM) loss, restoring SOD-1 expression, suppressing MMP-9, and improving epidermal barrier function in D-galactose-treated mice.
- The ethyl acetate fraction (JM02102), and its active compound chlorogenic acid (CGA) demonstrated the most potent anti-glycation, antioxidant, and collagen-promoting activities, by effectively alleviating ECM degradation and enhancing epidermal barrier function.
- Both JM02102 and CGA reverse skin aging features in glucocorticoid-treated mice by restoring dermal and epidermal thickness, suppressing REDD1 and p16^{INK4A} expression, and improving epidermal barrier function, thus validating the plant's traditional use.

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In brief

This study reveals that the root extract of *Vicatia thibetica* (JM02001), the ethyl acetate fraction (JM02102) and its active compound CGA protect against skin aging *in vivo* by preserving extracellular matrix, decreasing oxidative stress, and enhancing epidermal barrier function, thus validating its traditional use.

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ABSTRACT

Vicatia thibetica de Boiss (*V. thibetica*), a traditional medicinal and edible plant used by the Bai ethnic group in China, is known for its effects in tonifying qi, nourishing the blood, dispelling dampness, and relieving itching. Previous studies have shown that its root extract (JM02001) exerts anti-aging effects by promoting collagen expression and antioxidant potential. However, its anti-skin aging efficacy *in vivo* and its bioactive constituents have been largely unexplored. Herein, JM02001 was found to mitigate extracellular matrix (ECM) loss, restore superoxide dismutase-1 (SOD-1) expression, suppress matrix metalloproteinase-9 (MMP-9) expression, and increased the epidermal barrier intensity in D-galactose-treated mice. The ethyl acetate fraction (JM02102) exhibited the most promising anti-glycation, antioxidant, and collagen-promoting activities. Chlorogenic acid (CGA) was identified as an important active compound. Both JM02102 and CGA alleviated ECM degradation and enhanced epidermal barrier function in D-galactose-treated mice. Furthermore, in glucocorticoid-treated mice, they restored dermal and epidermal thickness, suppressed REDD1 and p16^{INK4A} expression, and enhanced epidermal barrier function. Therefore, our findings indicated that the extract of *V. thibetica* root and its compound CGA protect against skin aging by preserving ECM, decreasing oxidative stress, and reinforcing the skin barrier, thus validating its traditional use and highlighting its potential as a source of anti-aging therapeutics.

Keywords: *Vicatia thibetica* de Boiss, skin aging, D-galactose, glucocorticoid

1. INTRODUCTION

Skin, the largest organ of the human body, is a crucial barrier between the internal systems and the external environment, as well as a complex physiological system involved in thermoregulation, metabolism, and sensory perception [1]. Skin aging is driven by both intrinsic factors (chronological changes) and extrinsic stressors such as ultraviolet (UV) radiation, air pollution, and nutritional deficiencies. These processes lead to the accumulation of advanced glycation end products (AGEs)

[2], oxidative damage [3], and loss of collagen [4], and ultimately result in decreased elasticity and tensile strength, chronic inflammation, and progressive extracellular matrix (ECM) deterioration in the epidermis and dermis [5,6].

D-galactose (D-gal), a potent glycosylation agent [7], is widely used to establish skin aging models, which effectively simulate many biological changes associated with human aging [8] including enhanced AGE formation [7], oxidative stress, and disruption of collagen and elastin integrity [9]. Topical glucocorticoid-induced skin atrophy

often manifests features similar to skin aging, such as epidermal and dermal thinning and impaired barrier function. Furthermore, elevated 11β -hydroxysteroid dehydrogenase in aged skin leads to increased endogenous glucocorticoid activity [10], and glucocorticoid treatment directly induces cellular senescence in human dermal fibroblasts [11]. These findings collectively indicate that glucocorticoid plays a major role in skin aging. Therefore, both D-gal- and glucocorticoid-induced skin aging models provide robust experimental tools for evaluating the anti-skin-aging effects of therapeutic drugs.

Vicatia thibetica de Boiss (*V. thibetica*) is a medicinal plant documented in traditional compendia such as the *Illustrated Handbook of Medicinal Plants of the Bai Ethnic Group* and *Annals of Yunnan Province*. It has been used by the Bai ethnic group in Yunnan, China, as a tonic to strengthen the spleen, nourish the stomach, boost qi, and enrich the blood. According to the 1993 *Directory of Medicinal Resources in Yunnan*, the medicinal part, the root, has properties described as pungent, sweet, slightly bitter, and warm, and effects including nourishing the body, eliminating dampness, and relieving itching. In line with these traditional uses, our previous study demonstrated that its root extract (JM02001) extends the lifespan and upregulates collagen expression in *Caenorhabditis elegans*, and increases type I collagen levels in human skin fibroblasts [12]. These results suggest the potential of *V. thibetica* in anti-skin-aging applications. However, its comprehensive efficacy and the active constituents responsible for these effects remain uncharacterized.

Therefore, this study (i) evaluated the anti-skin-aging effects and underlying mechanisms of JM02001 in a D-gal-induced aging model; (ii) isolated and screened its bioactive fractions, thereby identifying the ethyl acetate fraction (JM02102) as the most promising active constituents; (iii) characterized the important active compound in JM02102, chlorogenic acid (CGA), through high-performance liquid chromatography (HPLC) and ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS); and (iv) compared the anti-skin aging efficacy of JM02001, JM02102, and CGA in both D-gal- and glucocorticoid-induced mouse models of skin aging.

2. METHODS

2.1 Preparation of JM02001 extract

Roots of *V. thibetica* were collected in Machang Village, Heqing County, Dali Prefecture (100°4'13" E, 26°29'28" N) by Professor Conglong Xia from Dali University, and were identified by the Kunming Institute of Botany as a species belonging to the Apiaceae family and the genus *Vicatia*. JM02001 was prepared and purified as previously described [12]. Dried roots of *V. thibetica* (10 kg) were crushed with a shredder, then decocted with a 10× volume of distilled water three times under continuous stirring (80°C, 120 minutes). The water extract was

concentrated by rotary evaporation and lyophilization to obtain JM02001, weighing 4.55 kg, with a yield of 45.5%.

2.2 Extraction and isolation of constituents from JM02001

The sequential fractionation of JM02001 was performed as follows. First, JM02001 was initially dissolved in 100 mL deionized water and mixed with 600 mL anhydrous ethanol (Aladdin, Shanghai, China) under continuous stirring, then centrifuged to separate the precipitate (JM02002) from the supernatant (JM02003). Then JM02002 was dried at 60°C to remove residual ethanol and subsequently lyophilized to obtain the final dry powder, and JM02003 was evaporated in a rotary evaporator under decreased pressure. Subsequently, JM02003 was extracted with petroleum ether, ethyl acetate, and n-butanol (Aladdin, Shanghai, China) successively three times. All extracts were evaporated, thus yielding JM02101, JM02102, and JM02103, respectively. The raffinate layer (JM02104) was finally lyophilized. The yields of JM02002, JM02003, JM02101, JM02102, JM02103, and JM02104 were recorded as 52.41%, 45.89%, 0.02%, 1.24%, 2.69%, and 18.33%, respectively. All solvents were of HPLC grade and obtained from Greagent (Shanghai, China).

2.3 Anti-glycation capacity assays

To evaluate the anti-glycation capacity of JM02001 and its isolated constituents, we incubated each sample with bovine serum albumin (BSA) (Lablead, Beijing, China) in PBS. Next, the glycation reaction of BSA was triggered by methylglyoxal (MGO) (Novus Biologicals, Littleton, USA). Aminoguanidine sulfate (AGS) (Macklin, Shanghai, China) at 1 mg/mL was used as a positive control [13]. The blank control comprised the reaction system without MGO, test samples, or AGS, whereas the negative control contained only MGO without samples or AGS. All reaction mixtures were incubated at 37°C in the dark for 72 h. After incubation, BSA was separated with SDS-PAGE electrophoresis and transferred onto 0.22 µm nitrocellulose (Millipore, Massachusetts, USA) membranes. AGEs were quantified by measurement of membrane autofluorescence at excitation wavelengths of 365 nm and 488 nm. The membranes were stained with Ponceau S as a loading control. The membranes were then blocked in 5% non-fat milk at room temperature for 1 hour and incubated with antibodies to N ϵ -carboxymethyl-lysine (CML) (R&D Systems, Minneapolis, USA) at 4°C for 12 hours. After being washed, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Yeasen, Shanghai, China) and reacted with 3,3'-diaminobenzidine (DAB) (Servicebio, Wuhan, China) in the dark for 5 minutes. Staining intensity was quantified to assess glycation inhibition.

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2.4 Determination of total phenolic and flavonoid content

The polyphenol and flavonoid content in each fraction was quantified with established spectrophotometric methods. The content of polyphenols was determined with the Folin-Ciocalteu method [14] and reported in gallic acid (GAE, Macklin, Shanghai, China) equivalents (μg GAE/mg sample). Similarly, total flavonoid content was measured with aluminum chloride colorimetric assays [15] and reported in rutin (RE, Macklin, Shanghai, China) equivalents (μg RE/mg sample). For both assays, standard curves were generated with serial dilutions of GAE and RE.

2.5 DPPH and ABTS free radical scavenging assays

The compounds 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Macklin, Shanghai, China) are frequently used to measure the free radical scavenging activity of antioxidants [16]. Various concentrations of vitamin C (Meryer, Shanghai, China) were used to construct a standard curve. In DPPH assays, 350 μL JM02001-JM02003 and JM02101-JM02104 were dissolved in methanol. The mixture was then centrifuged, and the supernatants were mixed with 350 μL of 100 μM DPPH solution and incubated in the dark at room temperature for 30 minutes. The mixture was centrifuged to remove insoluble particles, and only the supernatant was added to a 96-well plate. The absorbance was measured at 517 nm with a microplate reader. In ABTS (Beyotime, Shanghai, China) assays, the reaction system was prepared with the same procedure as in the DPPH assay, with methanol replaced by 80% ethanol. After thorough mixing, the reaction mixture was maintained at room temperature for 5 minutes away from light, then added to a 96-well plate. The absorbance was measured at 734 nm with a microplate reader.

2.6 Offline HPLC-MGO assays

JM02102 (2 mg/mL) dissolved in PBS was mixed with the same volume of PBS or 100 mM MGO (Macklin, Shanghai, China), and the samples were kept at 37°C for 24 hours. Samples (10 μL) were injected into an HPLC instrument (Waters, Milford, MA, USA) with a Unitray C18 chromatographic column (150 $\times$ 4.6 mm i.d., 5.0 μm , 100 Å) (Acchrom, Taizhou, China), with 0.1% (v/v) formic acid (Macklin, Shanghai, China) and acetonitrile as the mobile phase (flow rate: 1 mL/min; elution gradient: 0.0–30.0 minutes, 10%–90% acetonitrile). The spectra were recorded at 327 nm. Waters Empower 3 was used for data collection [17].

2.7 Offline HPLC-DPPH assays

JM02102 (2 mg/mL) dissolved in methanol was mixed with the same volume of methanol or 800 μM DPPH, and the samples were kept at room temperature for 30 minutes away from light. The detection conditions were the same as those in the HPLC-MGO analysis [18].

2.8 UPLC-MS/MS assays

Samples were analyzed with ultra-performance liquid chromatography-tandem Q-Exactive quadrupole-electrostatic field trap high-resolution mass spectrometry (UPLC-Q-Exactive) (Thermo Fisher Scientific, Massachusetts, USA) and a Zorbax Eclipse Plus C18 (C18, 100 mm $\times$ 2.1 mm, 1.8 μm) (Agilent, California, USA) chromatographic column at 35°C. The mobile phase consisted of A 0.1% (v/v) formic acid and B acetonitrile, and the run was programmed as follows: 0.0–20.0 minutes, 10%–50% B; 20.0–25.0 minutes, 50%–80% B; 30.0–30.1 minutes, 90%–100% B; and 30.1–35.0 minutes, 100% B. The sample concentration was 0.05 mg/mL, and the injection volume was 5 μL . Quality analysis of effluent was performed with Q-Exactive Plus, and MS was performed and recorded by electrospray ionization in negative-ion mode. The mass range was 100.00–1500.00 m/z . MS2 was performed with high-energy collision dissociation and analyzed in Thermo Xcalibur software.

2.9 Cell culture

Human foreskin fibroblasts (HFFs) were obtained from Cell Bank, Chinese Academy of Sciences, and cultured in DMEM (Thermo Fisher Scientific, Massachusetts, USA) supplemented with 15% fetal bovine serum (Thermo Fisher Scientific, Massachusetts, USA) and 1% penicillin/streptomycin (Yeasen, Shanghai, China). All cells were cultured at 37°C in a humidified atmosphere of 95% air and 5% CO_2 . Young HFFs (<30 passages) were used.

2.10 Cell viability assays

HFFs were seeded in 96-well plates at a density of 8,000 cells per well and cultured overnight. The cells were then treated with various concentrations of JM02001, JM02002, JM02003, JM02101, JM02102, JM02103, JM02104, or CGA (0, 10, 20, 40, 80, 160, or 320 $\mu\text{g}/\text{mL}$) for 72 hours. After treatment, cell viability was assessed with CCK-8 assays (TargetMol, Massachusetts, USA), in which 100 μL 10% CCK-8 solution was added to each well and incubated at 37°C for 2 h. The absorbance was measured at 450 nm with a microplate reader (Synergy H1) (BioTek, Winooski, USA).

2.11 Animal treatment

Seven-week-old female C57BL/6J mice (Charles River, Massachusetts, USA) were maintained in specific-pathogen-free facility laboratory conditions with stable, moderate temperature and humidity and remained untreated for 1 week before the experiments. Animal handling and procedures were performed in accordance with National Institutes of Health guidelines and approved by the ethical committee of Dali University (contract No. MECDU-202410-26).

Effects of JM02001 on D-gal-induced skin aging: A total of 30 mice were randomly divided into five groups of six animals each: a control group, a D-gal model group, and three JM02001-treated groups administered

low, moderate, or high doses. For 8 successive weeks, the mice in the control group and the other groups were treated with daily subcutaneous injection of normal saline or 1 g/kg D-gal (Macklin, Shanghai, China), respectively. Meanwhile, the mice were administered drinking water (control group and D-gal group) or different concentrations of JM02001 (0.6 g/kg, 1.2 g/kg, or 2.4 g/kg) dissolved in the drinking water daily for 8 weeks. All mice were sacrificed on day 56, and the back skin was isolated for subsequent experiments.

Effects of JM02102 and CGA on D-gal-induced skin aging: A total of 36 mice were randomly assigned to six groups of six animals each: a control group, a D-gal model group, two JM02102-treated groups (low or high dosage), two CGA-treated groups (low or high dosage), and a vitamin C positive control group. The administration of D-gal was as described above. Except in the control group and D-gal group, the mice were administered JM02102-L (45 mg/kg), JM02102-H (90 mg/kg), CGA-L, or CGA-H (90 mg/kg) dissolved in drinking water daily for 8 weeks. All mice were sacrificed on day 56, and the back skin was isolated for subsequent experiments.

Effects of JM02001, JM02102, and CGA on glucocorticoid-induced skin aging: A total of 36 mice were randomly assigned to six groups of six animals each: a control group, a fluocinolone acetonide (FA) model group, a group treated with JM02001, a group treated with JM02102, a CGA-treated group, and a rapamycin positive control group. The hair on the backs of the mice was shaved 1 day before the application of 40% isopropanol or FA (Aladdin, Shanghai, China). The control group received 40% isopropanol, and the other groups received 10 µg/mL FA (dissolved in 40% isopropanol) applied to the back skin once every other day for 2 weeks. Meanwhile, the control group and FA group were administered drinking water, whereas the other groups were administered JM02001 (2.4 g/kg), JM02102 (45 mg/kg), or CGA (45 mg/kg) dissolved in drinking water daily for 3 weeks. All mice were sacrificed on day 21, and the back skin was isolated for subsequent experiments.

2.12 Western blot analysis

Protein was extracted from cells and mouse back skin with RIPA lysis buffer (Yeasen, Shanghai, China) supplemented with protease inhibitors. A BCA protein assay kit (Yeasen, Shanghai, China) was used to measure the concentrations of protein according to the manufacturer's protocol. Protein was mixed with loading buffer (Yeasen, Shanghai, China) and heated at 100°C for 10 minutes, then separated with SDS-PAGE gel electrophoresis and transferred to 0.22 µm nitrocellulose membranes. The membranes were subsequently blocked with 5% non-fat milk and incubated with primary antibodies at 4°C for 12 hours. After being washed, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies (Yeasen, Shanghai, China) and detected through enhanced chemiluminescence (Tanon,

Shanghai, China). ImageJ software was used to quantify protein expression.

2.13 Sirius red and Alcian blue staining

Mouse back skin was collected and fixed in 4% paraformaldehyde, embedded in paraffin, cut into 4 µm slices, and mounted onto slides. The skin paraffin sections were deparaffinized, rehydrated, and stained in Sirius red (Servicebio, Wuhan, China) for 1 hour or Alcian blue (Servicebio, Wuhan, China) for 12 hours at room temperature. The sections were then washed quickly in anhydrous ethanol, and the process was repeated three times to remove the remaining stain from the surrounding tissue. Finally, the sections were soaked in xylene for 5 minutes and sealed with neutral balsam (Beyotime, Shanghai, China). Collagen deposition was quantified in ImageJ software across multiple randomly selected fields per sample (at least five fields per section in three sections per group).

2.14 Toluidine blue staining

The skin paraffin sections were deparaffinized, rehydrated, and stained with 1% toluidine blue (Servicebio, Wuhan, China) for 3 minutes at room temperature, then washed with running water. The sections were then dried at 65°C for 10 minutes, soaked in xylene for 5 minutes, and sealed with neutral balsam.

2.15 Immunofluorescence staining

The skin paraffin sections were deparaffinized, rehydrated, and boiled in Tris-EDTA antigen retrieval buffer for 20 minutes. The sections were returned to room temperature, blocked in 5% goat serum (Servicebio, Wuhan, China) at room temperature for 1 hour, and incubated with primary antibodies at 4°C for 12 hours. The sections were then washed in PBS three times and incubated with secondary antibodies at room temperature for 1 hour. Finally, the sections were stained with Hoechst in the dark for 5 minutes and observed under an inverted fluorescence microscope (Nikon, Shinagawa City, Japan).

2.16 Statistical analysis

Statistical analyses were performed in GraphPad Prism 8.3.0, and biological assay data are expressed as means ± SEM. Group comparisons were analyzed with unpaired two-tailed Student's t-tests for two-group analyses and one-way ANOVA for multi-group datasets. All results represent at least three independent experimental replicates, and the threshold for statistical significance was defined as $p < 0.05$.

3. RESULTS

3.1 JM02001 alleviates D-gal-induced skin aging

Administration of D-gal, compared with the control, led to skin aging phenotypes characterized by marked ECM depletion in both the epidermis and dermis, fewer proliferative cells, and impaired epidermal barrier function [19]. To evaluate the potential anti-skin aging effects of

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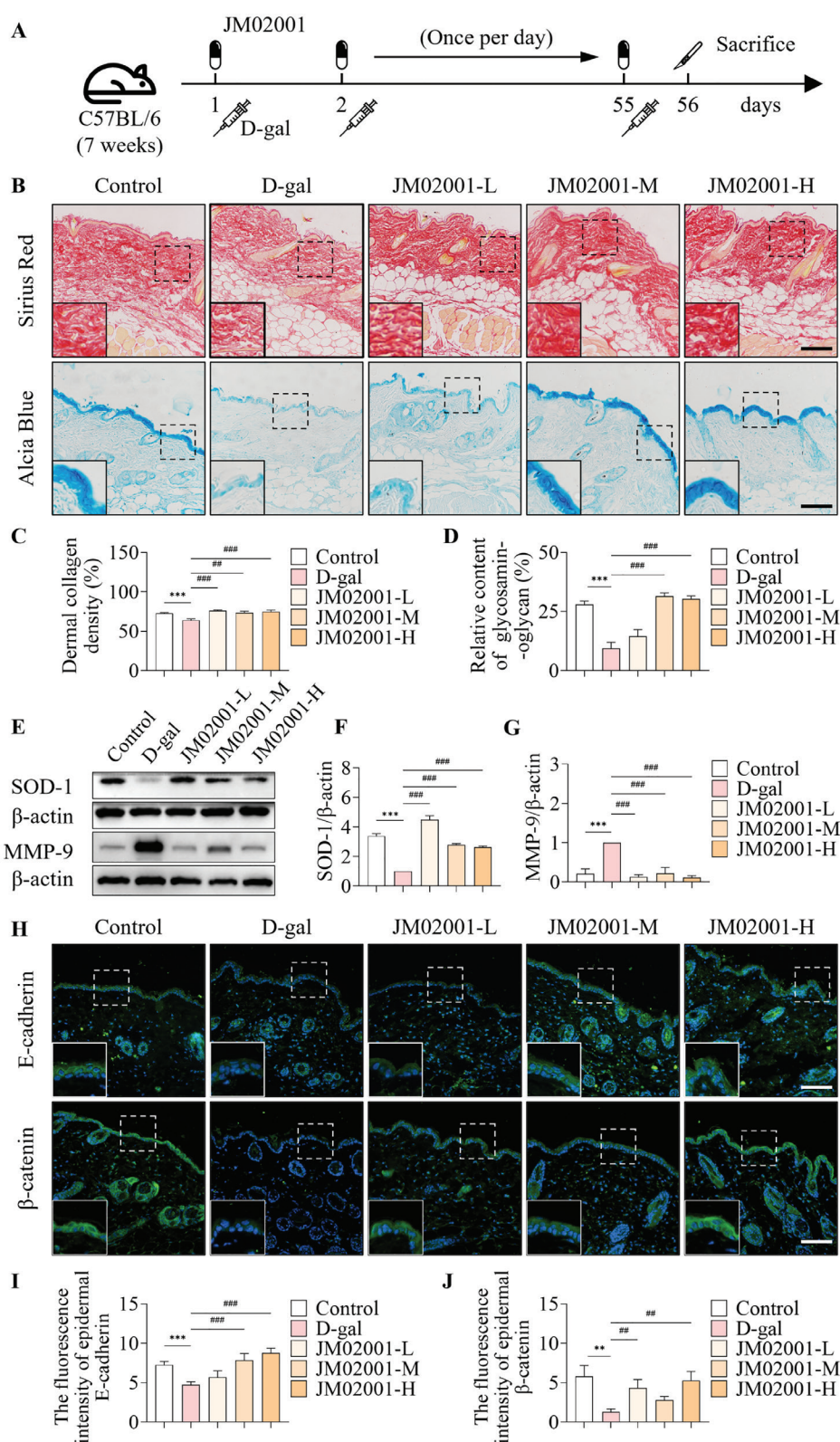


Figure 1 | JM02001 increases extracellular matrix content and enhances epidermal barrier function in a D-gal-induced skin aging mouse model.

(A) Schematic diagram of experimental design for the D-gal-induced skin aging mouse model. (B) Sirius red staining and Alcian blue staining

in mouse skin. Scale bar: 100 μ m. (C, D) Quantification of dermal collagen density and relative content of epidermal glycosaminoglycan. (E–G) Expression levels of SOD-1 and MMP-9 proteins in mouse skin. (H–J) E-cadherin and β -catenin immunofluorescence staining in mouse skin and quantification of fluorescence intensity. Scale bar: 100 μ m. All data are shown as mean $\pm$ SEM and compared with Student's t-test and one-way ANOVA. N = 3 per group. **p < 0.01, ***p < 0.001 vs. control; ##p < 0.01, ###p < 0.001 vs. D-gal.

JM02001 *in vivo*, we established a D-gal-induced skin aging model via subcutaneous injection of D-gal, followed by orally administered JM02001 at doses of 0.6, 1.2, or 2.4 g/kg, to assess its protective effects on skin (Figure 1A). In the D-gal group, Sirius red staining revealed 8.72% lower in dermal collagen density ($p < 0.001$), and Alcian blue staining indicated an 18.48% lower epidermal glycosaminoglycan content ($p < 0.001$) than observed in the control group (Figure 1B–D). JM02001 treatment effectively reversed these changes, by increasing collagen density by 12.01%, 9.45%, and 10.87% ($p < 0.01$) compared with D-gal group (Figure 1B, C) and by increasing glycosaminoglycan content by 22.13% and 20.98% at 1.2 and 2.4 g/kg, respectively ($p < 0.001$) (Figure 1B, D). The activity of superoxide dismutase (SOD) has been found to decrease in the skin after D-gal treatment [20], and SOD-1 deficiency causes oxidative stress, induces skin aging, and suppresses collagen transcription [21]. In addition, D-gal treatment increases the expression of matrix metalloproteinases (MMPs), key enzymes responsible for ECM degradation [22]. Western blot analysis further indicated that the 1.2 g/kg dose increased SOD-1 expression to 2.03-fold that in the control group ($p < 0.05$), whereas the three doses decreased MMP-9 expression to 0.29-, 0.46-, and 0.11-fold that in the D-gal group ($p < 0.05$) (Figure 1E–G), thus indicating enhanced antioxidant defense and ECM preservation.

The epidermis is the primary barrier against environmental insults; moreover, epidermal cell senescence directly affects barrier function, and often leads to inflammation and other skin diseases [23]. Immunofluorescence analysis showed that JM02001 increased the proportions of Ki67-positive epidermal cells by 7.32% and 5.69% at 1.2 and 2.4 g/kg, respectively ($p < 0.05$), and laminin B1-positive cells by 23.27% at 2.4 g/kg, respectively ($p = 0.056$) (Supplementary Figure 1A–C), thus suggesting enhanced proliferation and diminished senescence [24,25]. Furthermore, JM02001, compared with D-gal, significantly increased the epidermal expression of E-cadherin and β -catenin, proteins critical for barrier maintenance and downregulated in aged skin [26] (Figure 1H–J), thereby supporting enhanced epidermal barrier function.

Impaired epidermal barrier function promotes the accumulation of inflammatory immune cells, particularly eosinophils and mast cells [27]. Using mast cell protease 6 (MCPT6) as a mast cell marker, we observed that JM02001 at 0.6 and 2.4 g/kg led to an average of 7.23 and 7.61 fewer mast cells per field, respectively, than observed in the D-gal group ($p < 0.05$) (Supplementary Figure 1A, D), thus suggesting alleviation of aging-related inflammation.

Together, these findings indicated that JM02001 exerted multi-faceted protective effects in the D-gal-induced skin aging model, including ECM restoration, antioxidant enhancement, promotion of epidermal proliferation, enhancement in barrier function, and decreased inflammatory cell infiltration.

3.2 JM02102 is the main active constituent of JM02001

Given the demonstrated anti-skin aging effects of JM02001, we further investigated its active constituents. The graded extraction process of JM02001 is illustrated in Figure 2A. Each fraction was evaluated for anti-glycation, antioxidant, and collagen-promoting activities.

Anti-glycation capacity, antioxidant capacity, and collagen enhancement are critical indicators of anti-skin aging efficacy. The anti-glycation capacity of the isolated constituents of JM02001 was evaluated according to their inhibition of MGO-mediated BSA glycation, with AGS as a positive control [13]. Compared with AGE-BSA, JM02102 significantly decreased the fluorescence intensity at 365 nm and 488 nm, and additionally decreased anti-CML DAB staining, thus indicating potent anti-glycation capacity (Figure 2B). Polyphenols and flavonoids, known for their anti-AGE effects [28], were enriched in JM02102. As shown in Figure 2C, the Folin-Ciocalteu assay revealed a total phenolic content of 85.96 ± 0.42 μ g GAE/mg in JM02102 (standard curve: $y = 0.006174x + 0.07299$; $R^2 = 0.9973$; Supplementary Figure 2A). Similarly, the total flavonoid content, measured by aluminum chloride colorimetry, was 99.33 ± 3.22 μ g RE/mg (Figure 2D; standard curve: $y = 0.001937x + 0.05941$; $R^2 = 0.9985$; Supplementary Figure 2B).

Given the close relationship between glycation and oxidative stress [29], we assessed the antioxidant effects of isolated constituents of JM02001 with DPPH and ABTS assays. The vitamin C equivalent antioxidant capacity (VCEAC) was calculated for each constituent [16]. JM02102 exhibited the strongest scavenging effects on DPPH (15.94 ± 0.04 μ g/mg VCEAC) and ABTS (72.87 ± 1.77 μ g/mg VCEAC) (Figure 2E, F), thus indicating its optimal antioxidant capacity among all tested constituents (standard curves in Supplementary Figure 2C, D). Notably, JM02001 was a crude water extract containing high levels of sugars. During the preparation of JM02102 (ethyl acetate fraction), an ethanol precipitation step was used to remove sugars and selectively enrich phenolic and flavonoid compounds. This enrichment effect explains why JM02102 had a higher total phenolic content, total flavonoid content, and antioxidant capacity than JM02001 on a per-extract-weight basis.

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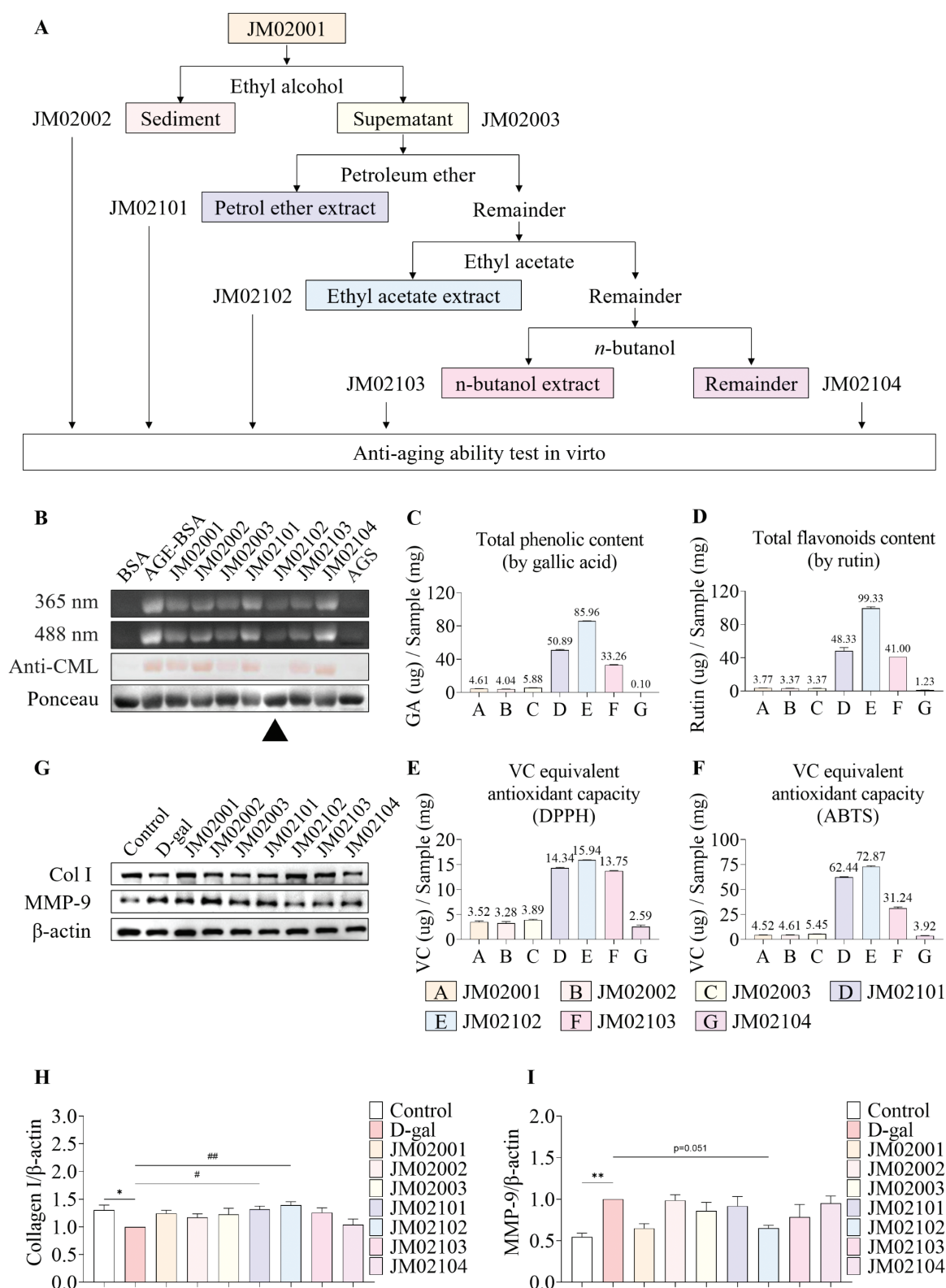


Figure 2 | JM02102, the main active constituent of JM02001, exhibits anti-glycation, and antioxidant and collagen accumulation.

(A) Graded extraction process of JM02001. (B) Comparative effects of JM02001 and its isolated constituents on the fluorescence intensity and

CML content of AGE-BSA. (C, D) Total phenolic and flavonoid content in JM02001 and its isolated constituents. (E, F). Vitamin C equivalent antioxidant capacity of JM02001 and its constituents, determined with DPPH and ABTS free radical scavenging assays. All values in C–F are expressed as mg/g extract dry weight. (G–I) Expression levels of Collagen I and MMP-9 proteins in HFFs after 72 h treatment. Data are shown as mean $\pm$ SEM and compared with one-way ANOVA. * $p < 0.05$, ** $p < 0.01$ vs. control; # $p < 0.05$, ## $p < 0.01$ vs. D-gal.

Given the decline in collagen with skin aging, we assessed collagen I and MMP-9 expression levels in HFFs treated with each fraction. Western blot analysis revealed that D-gal treatment decreased collagen I to 0.79-fold and increased MMP-9 to 1.82-fold the control levels ($p < 0.01$) (Figure 2H, I). Among all fractions, only JM02102 significantly increased collagen I expression, to 1.34-fold ($p < 0.001$), and decreased MMP-9, to 0.66-fold ($p < 0.001$) (Figure 2H, I), thereby indicating enhanced collagen synthesis and decreased degradation.

Together, these results demonstrated that the ethyl acetate fraction JM02102 was the most promising bioactive fraction of JM02001, and contributed to its anti-glycation, antioxidant, and collagen-promoting effects.

3.3 Compound analysis of JM02102 with HPLC-MGO, HPLC-DPPH, and UPLC-MS/MS

To identify the anti-glycation and antioxidant compounds in JM02102, we subsequently used offline HPLC-MGO and HPLC-DPPH assays, as previously described [17,18]. HPLC-MGO chromatograms before and after spiking with MGO (Figure 3A) revealed decreases in the peak areas of six compounds (G[1]–G[6]). Among them, compound G[1], with a retention time of 5 min 26 s, showed the largest decrease ($\Delta AU = 0.0079$) (Figure 3B) and therefore was considered the primary anti-glycation candidate in JM02102. Similarly, HPLC-DPPH analysis revealed six potential antioxidant compounds (O[1]–O[6]) in JM02102, which showed the largest peak area decreases after DPPH reaction (Figure 3C). Among these, compound O[2], with a retention time of 5 min 24 s, showed the largest decrease in peak area ($\Delta AU = 0.0293$) (Figure 3D) and therefore was considered the main antioxidant candidate in JM02102.

Analysis with UPLC-MS/MS in negative-ion mode ($m/z = 200,000$ – $1,500,000$) provided detailed total ion chromatograms and base peak chromatograms (BPC) (Figure 3E, F). Systematic interpretation of primary and secondary mass spectra (Supplementary Table S1) tentatively identified 13 bioactive compounds in JM02102, including multiple CGAs and derivatives. Peak G[1] (O[2]) corresponded to peak [3] in the BPC, which was characterized as CGA (3-*O*-caffeoylquinic acid, CGA) according to matching of retention times and MS2 fragmentation patterns against a CGA standard (Figure 3G–J).

Because JM02102 contains multiple CGAs and derivatives, among which CGA is the most representative, it was selected for further investigation. We compared the ability of CGA, JM02001, and JM02102 to promote collagen expression and decrease MMP-9 expression in HFFs after D-gal treatment. Western blot analysis indicated

that CGA, compared with D-gal group, significantly decreased MMP-9 expression to 0.65-fold ($p < 0.05$), whereas its effect on collagen I synthesis was minimal (Figure 3K–M). Therefore, CGA contributes to anti-aging effects primarily through MMP-9 inhibition rather than promoting collagen synthesis.

3.4 Both JM02102 and CGA alleviate D-gal-induced skin aging

The anti-skin aging effects of JM02102 and CGA were evaluated in a D-gal-induced skin aging model. JM02102 was administered at 45 or 90 mg/kg, and CGA was tested at equivalent doses for direct comparison. The treatment protocol followed that in the initial animal experiment (Figure 1A), and vitamin C (450 mg/kg) served as the positive control.

Sirius red staining indicated that dermal collagen density was 13.49% and 12.03% higher in the JM02102-L and JM02102-H groups, respectively, and 6.08% and 7.65% higher in the CGA-L and CGA-H groups, respectively, than the D-gal group ($p < 0.05$) (Figure 4A, B). Western blot analysis demonstrated significantly lower MMP-9 expression in the groups treated with various drug concentrations than the D-gal group (Supplementary Figure 3A, B). Immunofluorescence indicated that the percentage of Ki67-positive cells in the epidermis was 10.55%, 9.51%, 9.44%, and 5.39% higher in the four treatment groups than the D-gal group ($p < 0.01$) (Figure 4A, C). In addition, JM02102-H and CGA-L treatments resulted in significantly greater E-cadherin expression in the epidermis than observed in the D-gal group (Figure 4D, E). In contrast, all treatment groups showed significant upregulation of β -catenin expression (Figure 4D, F).

Collectively, JM02102 and CGA demonstrated anti-skin aging efficacy *in vivo*, by effectively mitigating dermal ECM degradation, enhancing epidermal proliferation, and strengthening barrier function. These results highlight their potential as therapeutic agents for skin aging.

3.5 JM02001, JM02102, and CGA alleviate glucocorticoid-induced skin aging

Topical glucocorticoids have been widely used in the treatment of various dermatological conditions including psoriasis and dermatitis. However, their clinical utility is markedly constrained by corticosteroid-induced skin aging manifestations such as skin atrophy and pigmentation [30]. To address this limitation, we systematically evaluated the protective effects of JM02001, JM02102, and CGA against glucocorticoid-induced skin aging. FA was applied every other day for 2 weeks to induce skin

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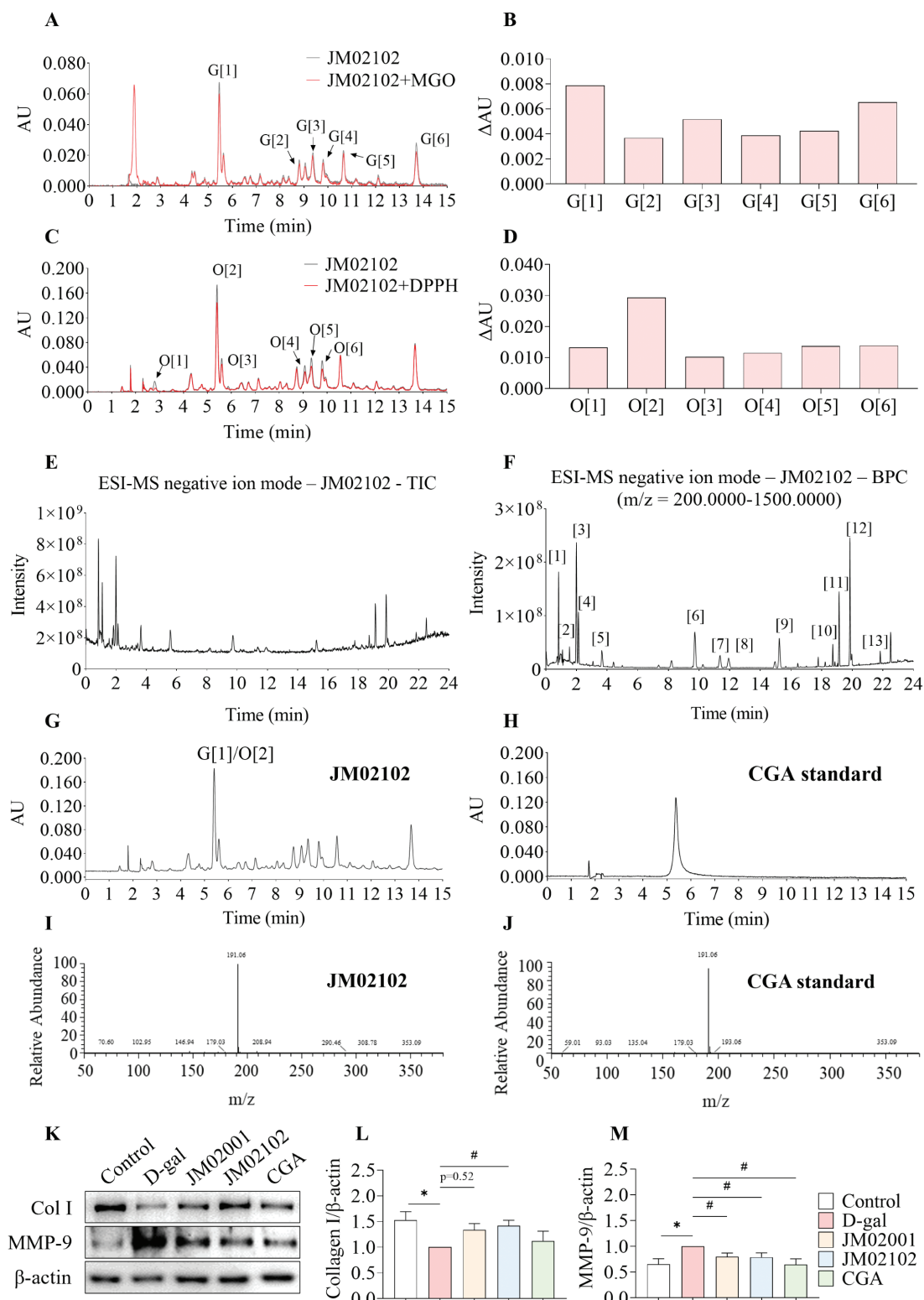


Figure 3 | CGA is the main anti-glycation and antioxidant substance in JM02102.

(A, B) Analysis of antiglycation components in JM02102 with offline HPLC-MGO and difference in HPLC response signals of G[1]–G[6] peaks before and after addition of MGO. JM02102 and JM02102 + MGO were analyzed on an Agilent e2695-2998 PDA system with a Unitray C18

chromatographic column (250 × 4.6 mm, 5 µm particle size). The mobile phases were 0.1% (v/v) carboxylic acid and acetonitrile at a flow rate of 1 mL/min. The effluent was detected with a 2998 photodiode array detector (PDA) and a monitoring wavelength of 327 nm. (C, D) Analysis of antioxidant components in JM02102 with offline HPLC-DPPH, and difference in HPLC response signals of O[1]–O[6] peaks before and after addition of DPPH. The experimental and testing conditions were as in (A). (E, F). Total ion current and base peak chromatogram of JM02102 in electrospray ionization negative-ion mode. (G, H). HPLC comparison of JM02102 with CGA standards at 327 nm. (I, J) Secondary mass spectrometry data comparison of JM02102 with CGA standards. (K–M) Expression levels of Collagen I and MMP-9 proteins in HFFs after 72 h treatment. Data are shown as mean ± SEM and compared with Student's t-test. N = 3 per group, *p < 0.05 vs. control; #p < 0.05 vs. D-gal.

atrophy, and was followed by daily administration of JM02001, JM02102, or CGA for 3 weeks (Figure 5A). Sirius red staining revealed that FA treatment significantly decreased the dermal thickness to 27.37% that in the control group ($p < 0.001$), whereas JM02001, JM02102, and CGA restored dermal thickness by 39.29%, 40.54%, and 32.45%, respectively ($p < 0.001$), thus outperforming the positive control rapamycin (RAPA) (Figure 5B, C). Toluidine blue staining demonstrated that FA decreased epidermal thickness by 32.42% ($p < 0.001$), whereas this effect was significantly reversed by JM02001 and CGA, which increased thickness by 24.56% and 28.78%, respectively ($p < 0.01$); however, JM02102 and RAPA showed no significant effect (Figure 5B, D). The limited effect of JM02102 might have been due to its low CGA content and the absence of other effective components.

Immunofluorescence analysis revealed significantly greater epidermal E-cadherin and β -catenin expression in all three treatment groups than the FA group (Figure 5E–G). Regulated in development and DNA damage response 1 (REDD1) has been identified as a key mediator of glucocorticoid-induced skin atrophy [31]. Western blotting revealed that REDD1 expression was upregulated to 1.85-fold in the FA group compared with the control group ($p < 0.05$), but was decreased to 0.56-, 0.54-, and 0.39-fold in the JM02001, JM02102, and CGA groups compared with the FA group, respectively ($p = 0.056$ or $p < 0.05$) (Figure 5H, I). The lack of REDD1 inhibition by RAPA might have been attributable to oral rather than topical administration. In agreement with previous findings [11], p16^{INK4A} levels were elevated to 2.35-fold in the FA group compared with the control group ($p < 0.05$), but were significantly decreased in the JM02001 and JM02102 groups compared with the FA group ($p < 0.05$) (Figure 5H, J).

Overall, JM02001, JM02102, and CGA attenuated glucocorticoid-induced dermal and epidermal atrophy, decreased cellular senescence, and restored epidermal barrier function. Our findings underscore their potential as protective agents against corticosteroid-induced skin aging.

4. DISCUSSION

This study provides the first systematic evidence, to our knowledge, that the root extract of *V. thibetica* (JM02001), its active fraction JM02102, and the identified compound CGA confer multi-faceted protection against skin aging by preserving the ECM, attenuating glycative and oxidative stress, and reinforcing the

epidermal barrier in two skin aging models induced by D-gal and glucocorticoid.

These findings provide a scientific rationale for the traditional use of *V. thibetica* de Boiss by the Bai ethnic group to tonify qi, nourish the blood, dispel dampness, and relieve itching. Specifically, the enhanced collagen synthesis and restoration of dermal structure offer a modern pharmacological correlate to the traditional “nourishing” effects associated with health and vitalized skin. The potent antioxidant and anti-glycation activities of JM02102 and CGA provide a molecular basis for the plant's documented function of “eliminating dampness,” a traditional concept often associated with the clearance of pathological metabolic accumulations, comparable in modern terms to the decrease in oxidative and glycation stressors. Additionally, the anti-aging potential of these components might offer mechanistic insight into the traditional application of relieving itching, given the established role of SASP in age-related pruritus. Furthermore, given the established role of the senescence-associated secretory phenotype (SASP) in age-related pruritus, the anti-aging potential of these components might offer mechanistic insight into the traditional application of relieving itching.

Screening of active fractions is a key step in exploring the material basis of traditional Chinese medicines. Through graded extraction of JM02001, the ethyl acetate fraction JM02102 exhibited the most significant anti-glycation, antioxidant, and collagen-promoting activities *in vitro* (Figure 2). Subsequent phytochemical analysis identified CGA as an important bioactive compound within JM02102, with potent anti-glycation and antioxidant activities (Figure 3A–J). Functional validation indicated that CGA effectively increased collagen I expression while suppressing D-gal-induced upregulation of MMP-9 (Figure 3K–M). These results align with prior reports demonstrating CGA's ability to enhance collagen production in dermal fibroblasts [32], decrease UVA-induced ROS accumulation, and suppress MMPs expression [33,34]. In the D-gal-induced skin aging model, both JM02102 and CGA reproduced the anti-aging effects of the parent extract JM02001, by significantly increasing collagen density, promoting epidermal proliferation, and enhancing barrier integrity (Figure 4). These findings were further corroborated by previous reports that CGA alleviates UV-B-induced photoaging *in vivo* by decreasing epidermal thickness, enhancing the collagen volume fraction, and suppressing glycosylation [35]. In addition, the lack of a clear dose-dependent response between 45 and 90 mg/kg for JM02102 and CGA is potentially

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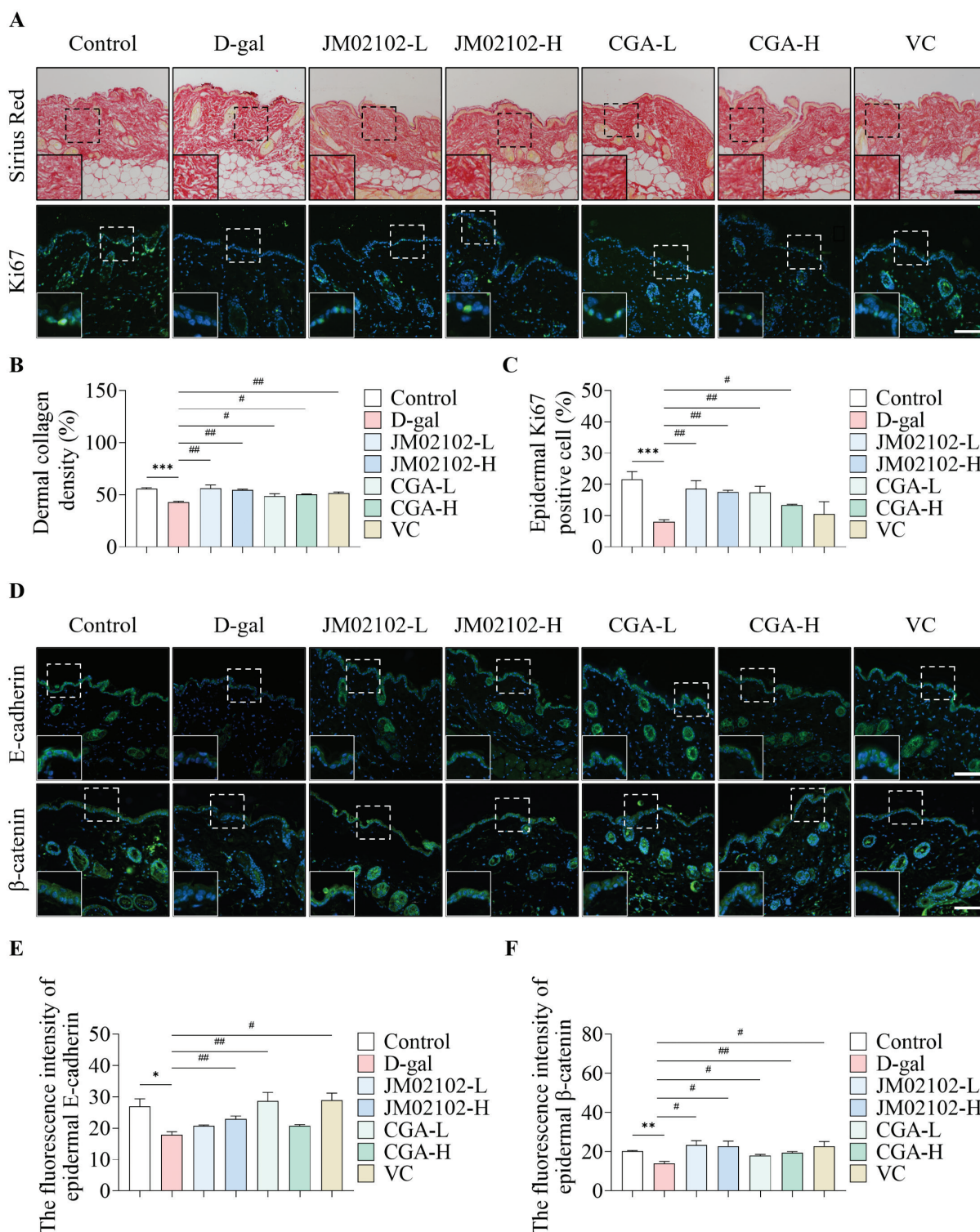


Figure 4 | JM02102 and CGA increase extracellular matrix content and enhance epidermal barrier function in a D-gal-induced skin aging mouse model.

(A) Sirius red staining and Ki67 immunofluorescence staining in mouse skin. Scale bar: 100 μ m. (B, C) Quantification of dermal collagen density and percentage epidermal Ki67-positive cells. (D–F) E-cadherin and β -catenin immunofluorescence staining in mouse skin and quantification of fluorescence intensity. Scale bar: 100 μ m. All data are shown as mean $\pm$ SEM and compared with Student's t-test and one-way ANOVA. N = 3 per group. * p < 0.05, ** p < 0.01, *** p < 0.001 vs. control; # p < 0.05, ## p < 0.01 vs. D-gal.

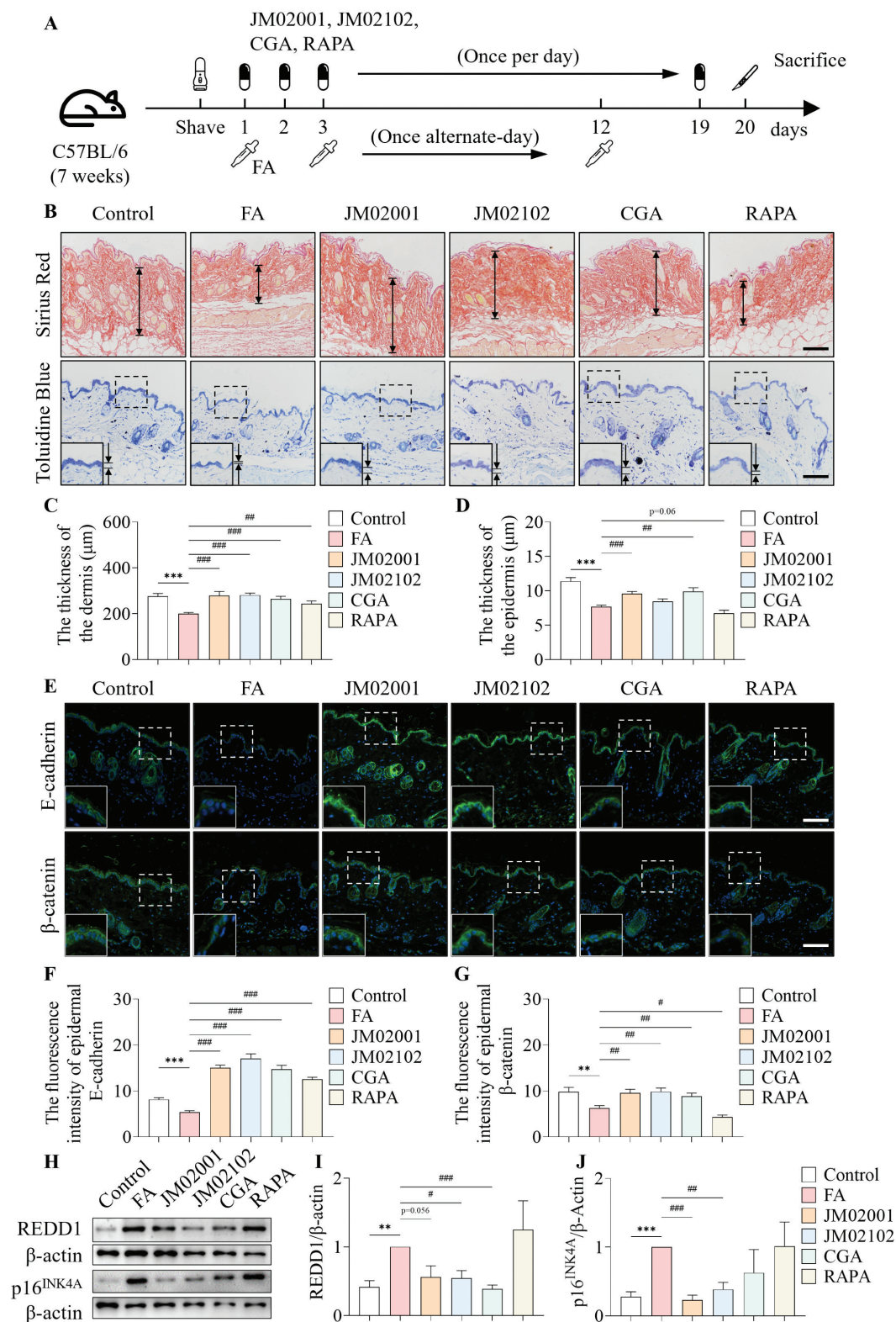


Figure 5 | JM02001, JM02102, and CGA attenuate skin atrophy in an FA-induced skin aging mouse model.

(A) Schematic diagram of experimental design for the FA-induced skin aging mouse model. (B) Sirius red staining and toluidine blue staining in mouse skin. Scale bar: 100 μ m. (C, D) Quantification of thickness of the dermis and epidermis. (E–G) E-cadherin and β -catenin

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immunofluorescence staining in mouse skin and quantification of fluorescence intensity. Scale bar: 100 μ m. (H–J) Expression levels of REDD1 and p16^{INK4A} proteins in mouse skin. All data are shown as mean $\pm$ SEM and compared with Student's t-test. N = 3 per group. **p < 0.01, ***p < 0.001 vs. Control; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. FA.

attributable to the relatively narrow dose interval tested, which might have been insufficient to resolve a clear dose–response relationship under the current experimental conditions. Alternatively, both concentrations might have been within near-maximal effective ranges, such that additional dosage increases did not further enhance the observed anti-aging or proliferative effects.

Notably, JM02001, JM02102, and CGA also showed remarkable protective effects against glucocorticoid-induced skin aging. JM02001 and CGA markedly restored dermal and epidermal thickness, whereas JM02102 predominantly increased dermal thickness (Figure 5B–D). This discrepancy might reflect the lower CGA content in JM02102 and the potential absence of other epidermis-active compounds present in JM02001. Similarly to their therapeutic effects in the D-gal-induced skin aging mouse model, JM02001, JM02102, and CGA also upregulated the expression of E-cadherin and β -catenin, thereby enhancing intercellular adhesion and strengthening the epidermal barrier (Figure 5E–G). Importantly, all treatments downregulated REDD1, a mediator of glucocorticoid-induced atrophy [31], as well as the senescence marker p16^{INK4A} [11] (Figure 5H–J), thus suggesting their broad anti-aging efficacy across distinct skin aging paradigms. To more convincingly support the claims regarding skin barrier enhancement and decreased cellular senescence, additional markers such as such as filaggrin or loricrin, SASP markers, or SA- β -gal activity should be included.

Although we identified JM02102/CGA as a bioactive fraction/component associated with changes in histological markers (collagen, glycosaminoglycans, and protein expression), we acknowledge that these findings are correlative and do not establish a causal mechanism for skin rejuvenation or barrier enhancement. Given the exploratory design of this study, our primary aim was to identify potential candidates and to characterize their correlation with structural markers of skin integrity. Future studies are warranted to elucidate the underlying mechanisms, including targeted functional assays and validation in human-relevant models, to establish the causal role of JM02102/CGA in skin functional outcomes.

5. CONCLUSION

In summary, the present study demonstrated that the root extract of *V. thibetica* (JM02001), its bioactive fraction JM02102, and CGA ameliorate skin aging through multiple converging mechanisms, including preservation of ECM integrity, suppression of oxidative stress, and enhancement of epidermal barrier function. The observed restoration of SOD-1 expression, alongside the downregulation of MMP-9, REDD1, and p16^{INK4A},

provided mechanistic support for these protective effects. These findings not only validate the traditional use of *V. thibetica*. As a skin-nourishing tonic in Bai ethnomedicine but also establish it as a promising botanical source for anti-aging interventions. Furthermore, the identification of CGA as an important active compound provides a phytochemical basis for its efficacy and a foundation for future development of standardized preparations. Further research is warranted to elucidate the precise molecular targets and signaling pathways involved, and to evaluate the translational potential of these natural agents in clinical settings.

ABBREVIATIONS

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); AGEs, advanced glycation end products; BPC, base peak chromatograms; BSA, bovine serum albumin; CGA, chlorogenic acid; D-gal, D-galactose; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ECM, extracellular matrix; FA, fluocinolone acetonide; GAE, gallic acid; HFFs, human foreskin fibroblasts; HPLC, high-performance liquid chromatography; MCPT6, mast cell protease 6; MGO, methylglyoxal; MMP-9, matrix metalloproteinases 9; RE, rutin; REDD1, regulated in development and DNA damage response 1; SOD-1, superoxide dismutase 1; TIC, total ion chromatograms; UPLC-MS/MS, ultra performance liquid chromatography tandem mass spectrometry; VCEAC, vitamin C equivalent antioxidant capacity.

DATA AVAILABILITY STATEMENT

All data and material will be made available upon request.

ETHICS STATEMENT

Animal handling and procedures were performed in accordance with National Institutes of Health guidelines and approved by the ethical committee of East China University of Science and Technology (ECUST-2025-134).

AUTHOR CONTRIBUTIONS

Daoux Zhang: Conceptualization, Methodology, Investigation, Data curation, Writing—original draft. Yunyu Hong: Methodology, Investigation, Data curation. Chengyi Wu: Methodology, Investigation, Data curation. Tianyue Sun: Methodology, Investigation, Data curation. Yehan Lu: Methodology, Data curation. Zhifan Mao: Methodology, Data curation. Chao Li: Validation. Yunta Zhang: Resources. Yongcheng Yang: Resources. Bo Han: Validation. Faliang An: Conceptualization, Methodology, Project administration, Writing—review & editing. Conglong Xia: Conceptualization, Methodology, Project administration, Writing—review & editing. Jian Li: Conceptualization, Methodology, Project administration, Writing—review & editing, Funding

acquisition. Zelan Hu: Conceptualization, Methodology, Project administration, Writing—review & editing, Funding acquisition.

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CONFLICT OF INTEREST

Jian Li is Deputy Editor-in-Chief of Acta Materia Medica. He was not involved in the peer-review or handling of the manuscript. The other authors have no other competing interests to disclose.

SUPPLEMENTARY MATERIAL

Supplementary Material can be downloaded from https://amm-journal.org/wp-content/uploads/2026/05/amm20260413_Suppl.pdf.

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