



Anti-skin-aging effects of tissue-cultured mountain-grown ginseng and quantitative HPLC/ELSD analysis of major ginsenosides

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Abstract

Mountain-grown ginseng has free radical scavenging activity and suppresses inflammation. We evaluated the anti-skin-aging effects of tissue-cultured mountain-grown ginseng (TG) and its major ginsenosides. The effect of three extracts of TG and ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) on the secretion of matrix metalloproteinase-1 (MMP-1) and collagen type I alpha 1 (COLIA1) was compared with that of tumor necrosis factor-alpha (TNF- α) stimulation of human dermal fibroblasts (HDFs), as determined via enzyme-linked immunosorbent assay. An analytical high-performance liquid chromatographic method with evaporative light-scattering detection (HPLC/ELSD) was developed for the simultaneous determination of the major ginsenosides in TG obtained via supercritical fluid CO₂ or ethanol extraction. TG residues obtained via supercritical fluid CO₂ extraction (TG1) and TG not subject to extraction (TG3) suppressed MMP-1 secretion in TNF- α -stimulated HDFs. Major ginsenoside content was higher in the TG1 than in residues extracted with ethanol (TG2) and TG3; ginsenoside Rg1 (1) content was the highest among all TG residues. Among them, ginsenosides Rg1 (1) and Re (4) suppressed MMP-1 in TNF- α -stimulated HDFs, whereas ginsenosides Rb1 (3) and Rd (5) increased COLIA1. In conclusion, TG and its active ginsenosides may have anti-skin-aging effects. Ginsenoside Rg1 (1) may also be beneficial in ameliorating skin damage. HPLC/ELSD can identify major ginsenosides and supercritical fluid CO₂ extraction can be applied during health supplement or drug development.

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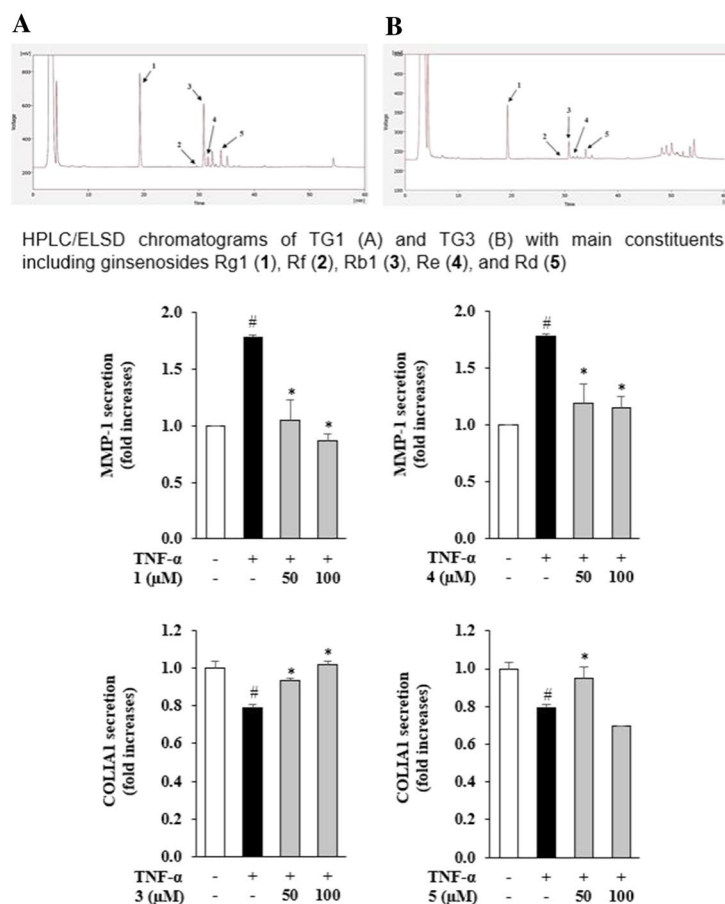
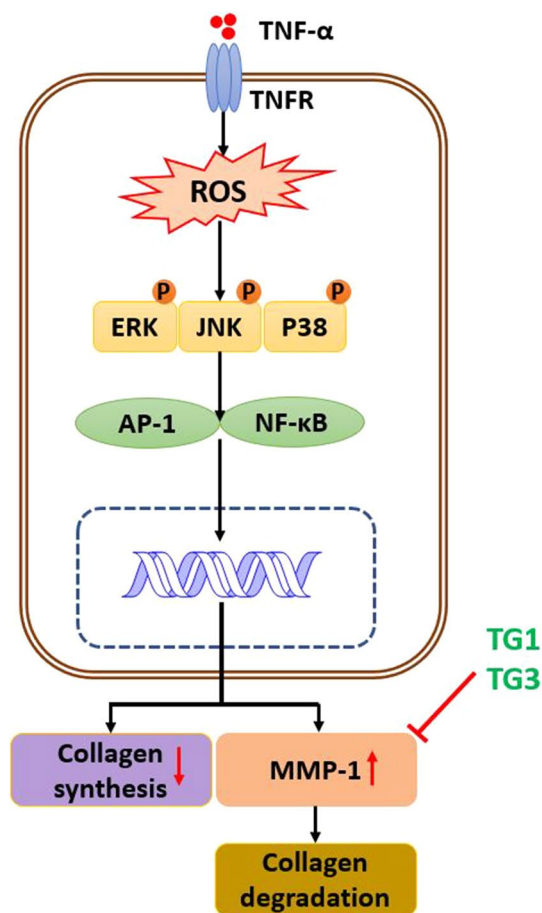
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Graphical abstract



Keywords Anti-skin-aging · HPLC/ELSD · Supercritical fluid CO₂ extraction · Tissue-cultured mountain-grown ginseng

Introduction

Skin is subject to natural aging the same as other organs of the human body. However, while the aging of inner organs cannot be observed by the naked eye, skin clearly shows multiple aging effects, including a rough-textured appearance, wrinkling, laxity, and poor elasticity. These effects are the result of the degradation of extracellular matrix components including elastin and collagen. In old skin, collagen appears disorganized and irregular, and the loss of collagen I results in an increase in the ratio of collagen III to collagen I [1, 2]. Reactive oxygen species (ROS) are generated from both the metabolism of cutaneous cells (intrinsic aging), as well as exogenous sources, including ultraviolet (UV) irradiation, chemicals, toxins, and pollutants (extrinsic aging). These exogenous factors are considered to be the primary factors promoting extracellular matrix depletion. ROS activate mitogen-activated

protein kinase transduction pathways leading to transcription and translation of matrix metalloproteinases (MMPs) that are involved in collagen breakdown [3, 4]. In addition to ROS, pro-inflammatory cytokines, including interleukin (IL)-1 β , IL-6, and IL-8 and tumor necrosis factor- α (TNF- α), arise in skin exposed to UV radiation and trigger inflammation-induced extracellular matrix degradation [5]. TNF- α secreted from keratinocytes and fibroblasts exposed to UV light is considered an important inflammation and oxidative stress regulator that promotes skin damage. Previous studies have demonstrated that TNF- α induces human dermal fibroblast damage through an increase in ROS production, and regulation of activator protein-1 and nuclear factor- κ B signaling pathways [5–7]. Oxidative stress and inflammation play key roles in skin aging. The reversal of these factors is one of the strategies for the delay or recovery of aged skin. Natural products are well-known as abundant and available antioxidant and anti-inflammatory sources. Furthermore, natural

compounds are safe and cause little irritation to the skin. Ancient populations used plants and herbs to ameliorate the effects of skin aging. For example, ancient Egyptians applied a mixture of olive oil, wax, incense cake, cypress, and fresh milk to soften and remove wrinkles [8].

Ginseng (*Panax ginseng* C. A. Meyer) is the root of a plant from the genus *Panax*. There are many types of ginseng, including mountain-grown ginseng and commercially processed ginseng, including red or white ginseng. Mountain-grown ginseng grows naturally in mountainous regions without human cultivation, grows slowly, and can take years to reach maturity. Because of the various bioactivities of mountain-grown ginseng, demand for ginseng health supplements has increased in recent years and, consequently, ginseng cultivation is increasing to meet market demands. Tissue-cultured mountain-grown ginseng (TG) has a better antioxidant effect than cultivated ginseng [9]. While TG has anti-obesity effects, mountain-grown ginseng herbal acupuncture has shown anti-cancer and anti-wrinkle effects [10–12] and TG is effective in preventing osteoporosis [13].

Ginsenosides are a class of steroid glycosides and triterpene saponins found almost exclusively in the genus *Panax*. Ginsenosides are divided into two groups: the dammarane family, which contains the most known ginsenosides, and the oleanane family. Dammaranes are further divided into protopanaxadiols and protopanaxatriols [14]. Protopanaxadiols include ginsenosides Rb1, Rb2, Rc, Rd, Rg3, and Rh2. Protopanaxatriols include ginsenosides Rg1, Rf, Re, and Rh1 [15]. Ginsenosides are denoted as Rx, where “R” stands for root and “x” stands for chromatographic polarity. Ginsenosides derived from ginseng possess multiple biological activities [16], including anti-cancer, cancer prevention, memory enhancement, immunomodulatory, and anti-platelet aggregation effects [17–20]. Ginseng contains a variety of active ingredients, including ginsenosides, polysaccharides, flavonoids, volatile oils, amino acids, and vitamins [21, 22]. Ginseng residues are also used as fertilizers in agriculture [23].

In this study, we evaluated the effect of TG and its major ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) on TNF- α -induced human dermal fibroblasts (HDFs) damage. Furthermore, we quantified major ginsenosides in different extractions of TG and validated analytical methods using high-performance liquid chromatography with evaporative light-scattering detection (HPLC/ELSD).

Materials and methods

Plant materials and cultivation of mountain-grown ginseng

TG were provided by Department of Farm Management, College of Agriculture and Life Sciences, Kyungpook National University, Daegu 41,566, Korea. Voucher

specimens were deposited at the herbarium of Department of Plant Science and Technology herbarium (Chung-Ang University, Anseong, Korea). Using the modified wood plant medium method, we added sugar (30 g/L) and indole butyric acid (pH 5.7) to the basic medium for mountain-grown ginseng cultivation [24, 25]. The medium was prepared in an 18 L bioreactor, sterilized at high temperature (125 °C) and high-pressure (0.15 MPa) for 45 min, and subsequently frozen. Cultivation was conducted at approximately 23 °C in a dark room for 6–7 weeks. Harvested ginseng was dried in hot air (45 °C) for 48 h and used as the TG for analysis.

Instruments and reagents

Chromatographic analysis was performed using an HPLC (Gilson 72 rue Gambetta—BP 45, 95,400, Villiers-le-Bel, France) equipped with a pump and ELSD. HPLC-grade water and acetonitrile were purchased from J. T. Baker (Avantor, Radnor, PA, USA). Other reagents including ethanol (EtOH) were analytical grade and purchased from Samchun Pure Chemicals (Pyeongtaek, Korea). Ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) were obtained from Natural Product Institute of Science and Technology (www.nist.re.kr), Anseong, Korea (Fig. 1).

Residues derived from different extraction methods and sample preparation

Crushed TG was extracted using supercritical fluid CO₂ extraction with a 1 L extraction tube at the R&D center, Phycoil Biotech Korea, Inc. A single batch of 300 g of each sample was extracted at 50 °C and 30 MPa for 8 h. An input of 162 g and recovery residue (154.3 g) was termed TG1. Crushed TG was also extracted with 95% EtOH for 5 h to obtain an extract (2.42 g); the residue after extraction was termed TG2 and the extraction yield was 1.49%. Untreated TG comprised TG3 (Fig. 2). For quantitative analysis of major ginsenosides, TG1–3 was extracted once more using the same method. TG1, TG2, and TG3 (7 g each) were extracted five times in EtOH (140 mL each) under reflux for 3 h. After extraction, samples were filtered and evaporated using a rotary evaporator at 50 °C to obtain the final extract. The extraction yields of TG1, TG2, and TG3 were 21.43%, 27.14%, and 27.14%, respectively.

Cell culture and treatment

HDFs were supplied by PromoCell GmbH (Sickingenstrasse, Heidelberg, Germany). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; R&D systems,

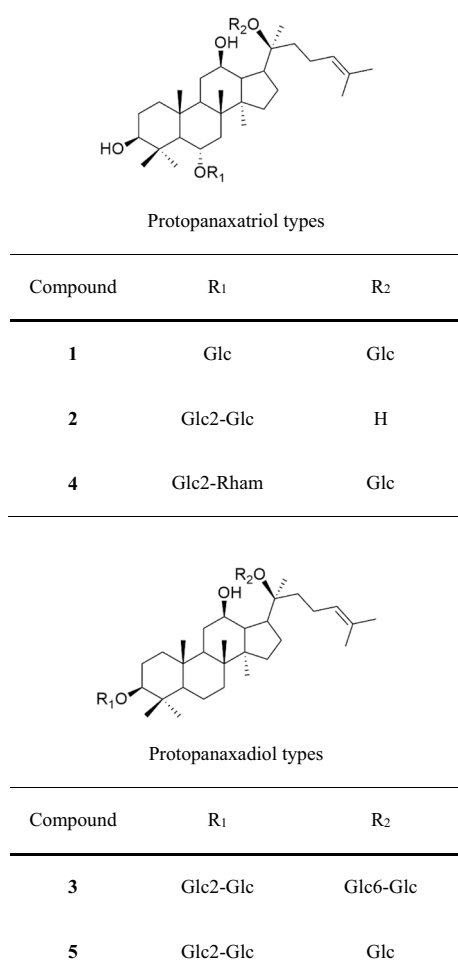


Fig. 1 Chemical structures of ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5)

Minneapolis, USA) supplemented with a 1% mixture of penicillin and streptomycin (Lonza, Basel, Switzerland) and 10% fetal bovine serum (FBS; R&D systems, Minneapolis, USA). HDFs were incubated at 37 °C, with 95% relative humidity, and 5% CO₂. Stock solutions of 100 mg/mL extracts (TG1, TG2, and TG3); 100 mM ginsenoside Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5); and 100 mM quercetin (Que) were prepared in dimethyl sulfoxide (DMSO;

Sigma-Aldrich, St. Louis, MO, USA). A 20 µg/mL stock of TNF-α (R&D Systems, Minneapolis, USA) was prepared in a 1% bovine serum albumin solution (BSA; Affymetrix, Cleveland, Ohio, USA). The final percentage of DMSO was controlled to less than 0.1%, so that the viability of HDFs compared to with non-treated cells, was not affected. For the experiments, HDFs were first pipetted into multi-well plates (3×10^4 cells/cm²) and allowed to adhere for 24 h. The cells were starved in serum-free DMEM for 24 h and then exposed to specific doses of samples for 1 h and subsequently to 20 ng/mL TNF-α.

Determination of cell viability

The HDFs were exposed to specific doses of the samples for 24 h in 96-well plates. EZ-Cytox solution (10 µL) (Dogen, Seoul, Korea) was then pipetted into each well and incubated for 30 min. The OD450 of the plates was assessed using a microplate reader (SPARK 10 M; Tecan, Männedorf, Switzerland). Cell viability was determined as a proportion of the viability of untreated cells.

Enzyme-linked immunosorbent assay (ELISA)

The cells were treated with known concentrations of the samples in the presence or absence of TNF-α in 48-well plates. After 24 h, HDFs were exposed to the indicated concentrations of samples for 1 h and subsequently to 20 ng/mL TNF-α for 24 h. Supernatants were then harvested and the concentrations of matrix metalloproteinase-1 (MMP-1) and collagen type I alpha 1 (COLIA1) were measured using a sandwich ELISA kit (R&D Systems, Minneapolis, USA).

Preparation of stock solutions for HPLC analysis

Experimental stock solutions were prepared by dissolving 25 mg of each extract in methanol (MeOH), sonicated for 20 min, and filtered using a 0.45-µm PVDF membrane filter. For the standard stock solutions, 1 mg of each standard was

Fig. 2 TG1 (A), TG2 (B), and TG3 (C)



dissolved in MeOH, sonicated for 20 min, and filtered using a 0.45 µm PVDF membrane filter.

HPLC conditions

Quantitative analyses of ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) were performed with a gradient elution HPLC system using a reverse-phase YMC pack Pro C18 column (4.6×250 mm, 5 µm). The injection volume was 20 µL, the temperature of the column was 35 °C, and the flow rate was 1 mL/min. The probe temperature of the ELSD was 75 °C and the nebulizer nitrogen gas flow was adjusted to 1.9 mL/min. The mobile phase consisted of water (A) and acetonitrile (B) and elution proceeded as follows: 83% A at 0 min, 65% A at 25 min, 55% A at 30 min, 55% A at 40 min, 100% B at 45 min, 100% B at 50 min, 83% A at 51 min, and 83% A at 60 min.

Method validation

The HPLC/ELSD method used to quantify major ginsenosides in TG was validated for precision, limit of detection (LOD), and limit of quantification (LOQ). We analyzed ginsenosides Rg1 (1) and Rb1 (3) as marker compounds in TG1. Triplicate calibration curves were plotted using chromatogram peak areas against the five concentrations of the standard solutions (7.8125–500 µg/mL) and the correlation coefficients were calculated by double logarithmic transformation of peak areas (Y) of the prepared concentrations (X). LOD and LOQ values were determined from the standard deviation (SD) of the calibration curve intercept (σ) and slope (S), with the following formulas: $\text{LOD} = 3.3 (\sigma/S)$ and $\text{LOQ} = 10 (\sigma/S)$. To assess precision, samples of three different concentrations (5, 7.5, and 12.5 mg/mL) were used for intra-day comparison, and a single concentration (7.5 mg/mL) for inter-day comparison. Determinations were repeated five times, and precision was expressed as the percentage relative standard deviation (% RSD) of the standard on three separate days and the same day.

Calibration curves

Standard stock solutions of ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) were prepared by dissolving in MeOH to yield a final concentration of 1 mg/mL. Working solutions used to construct the calibration curve were prepared by serially diluting the stock solutions. The calibration functions of ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) were calculated by the logarithmic transformation of the peak areas (Y) of the prepared concentrations (X, µg/mL), and the mean $\pm$ SD.

Statistical methods

Data are presented as means $\pm$ SD. Statistical differences were analyzed using one-way analysis of variance (ANOVA) with Tukey's honest significance test; *p*-values less than 0.05, 0.01 and 0.001 were considered statistically significant.

Results and discussion

We evaluated the anti-skin-aging effects of TG and its major ginsenosides in TNF- α -stimulated HDFs by attenuating MMP-1 secretion and increasing COLIA1 secretion. TG1 and TG3 suppressed MMP-1 secretion in TNF- α -stimulated HDFs. Quantitative analyses of major ginsenosides in TG1, TG2, and TG3 were performed using HPLC/ELSD. The content of each ginsenoside was higher in TG1 than in TG2 and TG3, with that of ginsenoside Rg1 (1) being significantly higher.

Ginseng and mountain-grown ginseng have been used most as medicinal crops to produce traditional Chinese or Asian medicines. Ginseng prevents or treats cancer, diabetes, osteoporosis, and oxidation [26–29]. Ginsenosides exert antioxidant, anti-cancer, and immunostimulatory pharmacological effects and can serve as potent anti-inflammatory agents for the prevention and treatment of various inflammatory diseases [30]. Ginseng and its constituents exert anti-skin-aging effects by suppressing enzymes, including MMPs, related to extracellular matrix degradation, prevention of skin inflammation, and stimulation of collagen biosynthesis [31, 32]. For example, sun ginseng is effective in promoting procollagen type I C-peptide generation by inhibiting MMP-1 and enhancing the expression of a tissue inhibitor of MMP-1 in normal HDFs [33]. Thus, TG and its components may have the potential to prevent skin aging. In this study, we demonstrated the effects of TG1, TG2, and TG3 on TNF- α -induced normal HDFs damage. Que was used as the positive control [34].

To determine the appropriate dose range for experiments, the effect of the test samples on the viability of HDFs was assessed. TG1, TG2, TG3, and Que were not cytotoxic to HDFs within the concentration range applied (Fig. 3A). Thus, we used 50 and 100 µg/mL TG1, TG2, and TG3 for subsequent assays. We evaluated the effects of TG1, TG2, and TG3 samples on TNF- α -stimulated HDFs using two markers: MMP-1 and COLIA1. Following treatment with 20 ng/mL TNF- α , MMP-1 secretion was significantly increased in the absence of TG1, TG2, and TG3, and Que, but decreased in groups exposed to TG1 and TG3 (Fig. 3B). TG2 did not show an inhibitory effect on TNF- α -stimulated

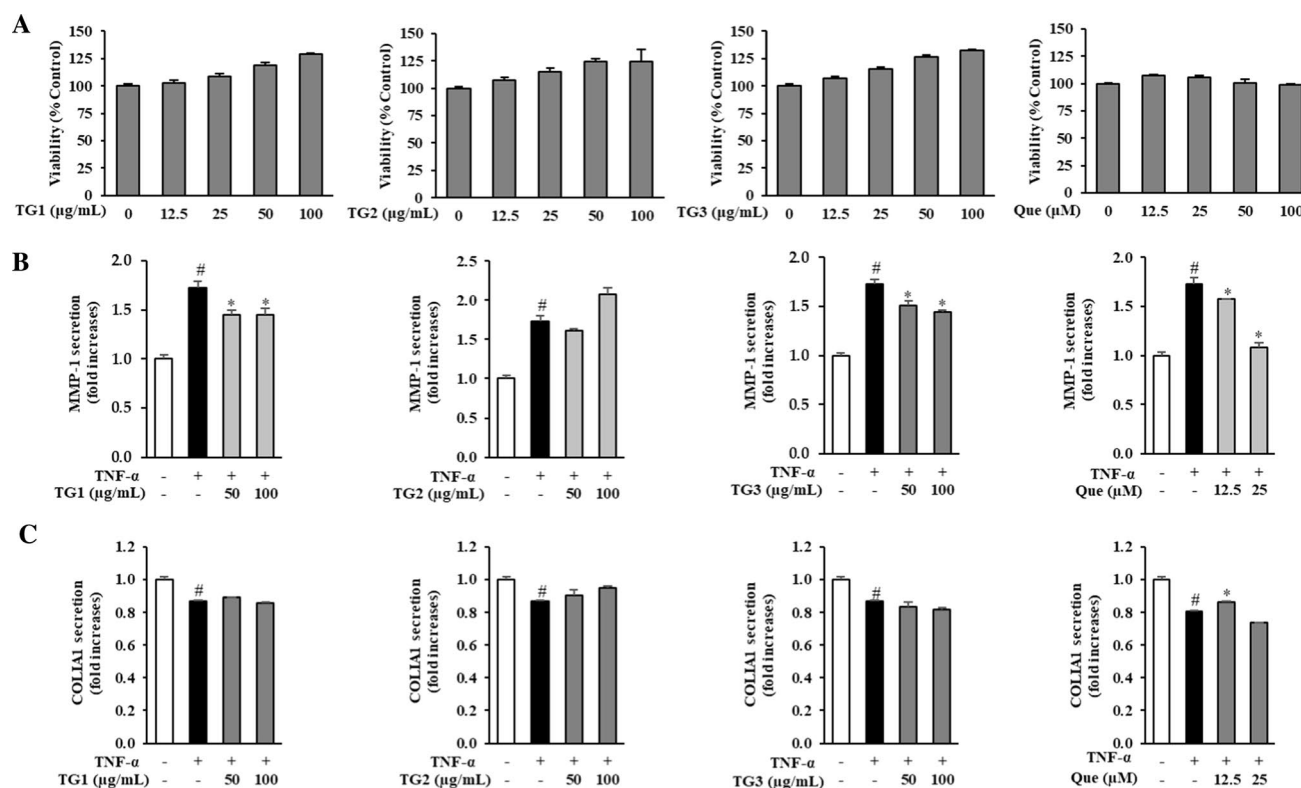


Fig. 3 Effect of TG1, TG2, and TG3 extracts and Que on cell viability (A), MMP-1 (B) and COLIA1 secretion (C) of HDFs. The viability of cells was assessed using EZ-Cytox kit. The

content of MMP-1 and COLIA1 in the supernatant was determined using an ELISA kit. Data are presented as the mean $\pm$ SD ($n=3$). # $p < 0.05$ vs. non-treated cells, * $p < 0.05$ vs. TNF- α -treated cells

MMP-1 secretion in HDFs. In addition, treatment with 20 ng/mL TNF- α considerably reduced COLIA1 secretion compared with the control group (Fig. 3C). TG2 increased the secretion of COLIA1 but the difference was not significant. TG1 and TG3 did not reverse the reduction in COLIA1 secretion induced by TNF- α in HDFs. Que as a positive control significantly recovered COLIA1 secretion at 12.5 μ M (Fig. 3C). These data suggest that TG1, TG2, and TG3 may prevent skin aging induced by TNF- α in HDFs via the inhibition of MMP-1 secretion.

Quantitative analysis of major ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) in TG1, TG2, and TG3 was performed using HPLC/ELSD with a reverse-phase column and a gradient elution with water and acetonitrile in the mobile phase. The linear correlation coefficients (r^2) for the standards ranged from 0.9990 to 0.9996. The chromatographic peaks of ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) exhibited good separation with retention times of 19.2, 29.4, 30.5, 31.3, and 33.7 min, respectively (Fig. 4). HPLC chromatograms of ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5), and the TG extracts are shown in Fig. 4. Table 1 shows the contents of the five ginsenosides in TG1, TG2, and TG3; the content of each ginsenoside was higher in TG1 than in TG2 and TG3, with that

of ginsenoside Rg1 (1) being significantly higher. The total ginsenoside content was highest in TG1, followed by TG2 and TG3. These results show the efficiency of supercritical fluid CO₂ extraction for yielding ginsenosides. Supercritical fluid CO₂ extraction is usually limited to the extraction of relatively non-polar compounds [35]. In our experiment, we analyzed the residues after extraction with supercritical fluid CO₂. Ginsenosides are generally polar and their high content in TG1 could be because of their retention in the residue, as most of the non-polar compounds were already excluded by supercritical fluid CO₂ extraction.

In method validation as marker compounds [36], we constructed calibration curves of ginsenosides Rg1 (1) and Rb1 (3) using five different concentrations. A linear regression equation and the correlation coefficient ($r^2 = 0.9991$ and 0.9994) were calculated by double logarithmic transformation of the peak areas (Y) of the prepared concentrations (X, μ g/mL). The LOQ values of ginsenosides Rg1 (1) and Rb1 (3) were 0.002 and 0.265 μ g/g, respectively. (Table 2). We evaluated analytical precision by performing intra- and inter-day measurements of samples. The RSD values for the intra- and inter-day precision values for ginsenoside Rg1 (1) ranged from 0.95% to 1.39% and from 0.49% to 0.87%, respectively, while corresponding values for ginsenoside

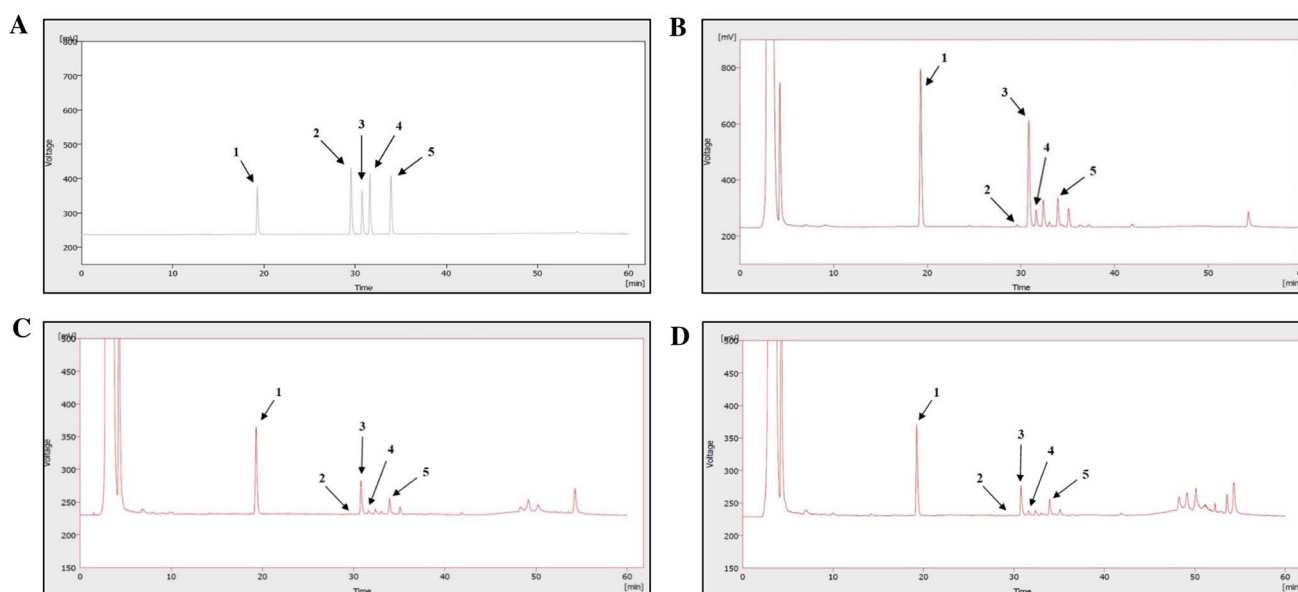


Fig. 4 HPLC/ELSD chromatograms of ginsenosides Rg1 (**1**), Rf (**2**), Rb1 (**3**), Re (**4**), and Rd (**5**) (A), TG1 (B), TG2 (C), and TG3 (D)

Table 1 The content of ginsenosides Rg1 (**1**), Rf (**2**), Rb1 (**3**), Re (**4**), and Rd (**5**) in TG1, TG2, and TG3

Compound	TG1		TG2		TG3	
	(mg/g extract)	(mg/g DW)	(mg/g extract)	(mg/g DW)	(mg/g extract)	(mg/g DW)
1	16.485 ± 1.624	3.533 ± 0.348	4.804 ± 0.231*	1.304 ± 0.063*	5.037 ± 0.333*	1.367 ± 0.090**
2	0.479 ± 0.015	0.103 ± 0.003	*trace***	*trace***	*trace***	trace***
3	6.127 ± 0.665	1.313 ± 0.143	1.291 ± 0.097*	0.350 ± 0.026*	1.241 ± 0.040*	0.037 ± 0.011**
4	1.829 ± 0.121	0.392 ± 0.026	*0.858 ± 0.007*	*0.233 ± 0.002*	*0.875 ± 0.024*	0.237 ± 0.006**
5	2.919 ± 0.264	0.625 ± 0.056	*1.297 ± 0.444**	*0.352 ± 0.012**	*1.347 ± 0.031**	0.366 ± 0.009**

Data are presented as mean ± SD ($n=3$) in mg/g. ** $p < 0.01$ and *** $p < 0.001$ compared with TG1

Table 2 Linearity, LOD, and LOQ values obtained for ginsenosides Rg1 (**1**) and Rb1 (**3**)

Compound	t_R (min)	Range (μg/mL)	Calibration equation ^a	r^2 ^b	LOD (μg/mL)	LOQ (μg/mL)
1	19.2	31.25–500	$Y = 2.1469X - 3.6116$	0.9991	0.001	0.002
3	30.5	7.8125–500	$Y = 1.5729X + 0.059$	0.9994	0.088	0.265

^a Y = log-transformed value of peak area, X = log-transformed value of concentration of the standard (μg/mL)

^b r^2 = correlation coefficient for five data points in the calibration curve

t_R = retention time

Rb1 (**3**) ranged from 1.13% to 1.55% and from 0.71% to 1.22%, respectively (Table 3). The fact that these RSD values were lower than 2% indicates the reliability of the analytical method for quantifying major ginsenosides.

There are several studies on the separation of major ginsenosides using reversed-phase HPLC [37, 38] and we were also able to successfully analyze five major ginsenosides using this technique. Lee et al. [39] quantified the major ginsenosides in ginseng roots of different ages, from different regions and countries, grown using different cultivation

practices. They demonstrated that the content of the major ginsenosides was high (0.931 mg/g) in ginseng cultivated in Jinan and the highest (1.785 mg/g) in 4 year-old red ginseng. Ok et al. [40] quantified six major ginsenosides in TG and demonstrated that the contents of ginsenosides Rg1 (**1**) and Rb1 (**3**) in their extracts were 12.80 and 5.48 mg/g, respectively. The contents of ginsenosides Rg1 (**1**) and Rb1 (**3**) in TG1 reported in the current study, were higher than that in the previous study, whereas the contents of ginsenosides Rf (**2**) and Re (**4**) reported in this study were lower.

Table 3 Intra- and inter-day precision for determining ginsenosides Rg1 (**1**) and Rb1 (**3**) in TG1

Compound	Intra-day (<i>n</i> = 5)			Inter-day (<i>n</i> = 5)		
	Concentration (mg/mL)	Measured content (mg/g)	RSD (%)	Concentration (mg/mL)	Measured content (mg/g)	RSD (%)
1	5	16.715	1.12	7.5	16.792	0.77
	7.5	16.786	0.95		16.814	0.87
	12.5	15.996	1.39		16.765	0.49
3	5	6.395	1.13	7.5	6.905	0.89
	7.5	6.890	1.25		6.904	1.22
	12.5	6.382	1.55		6.888	0.71

RSD relative standard deviation

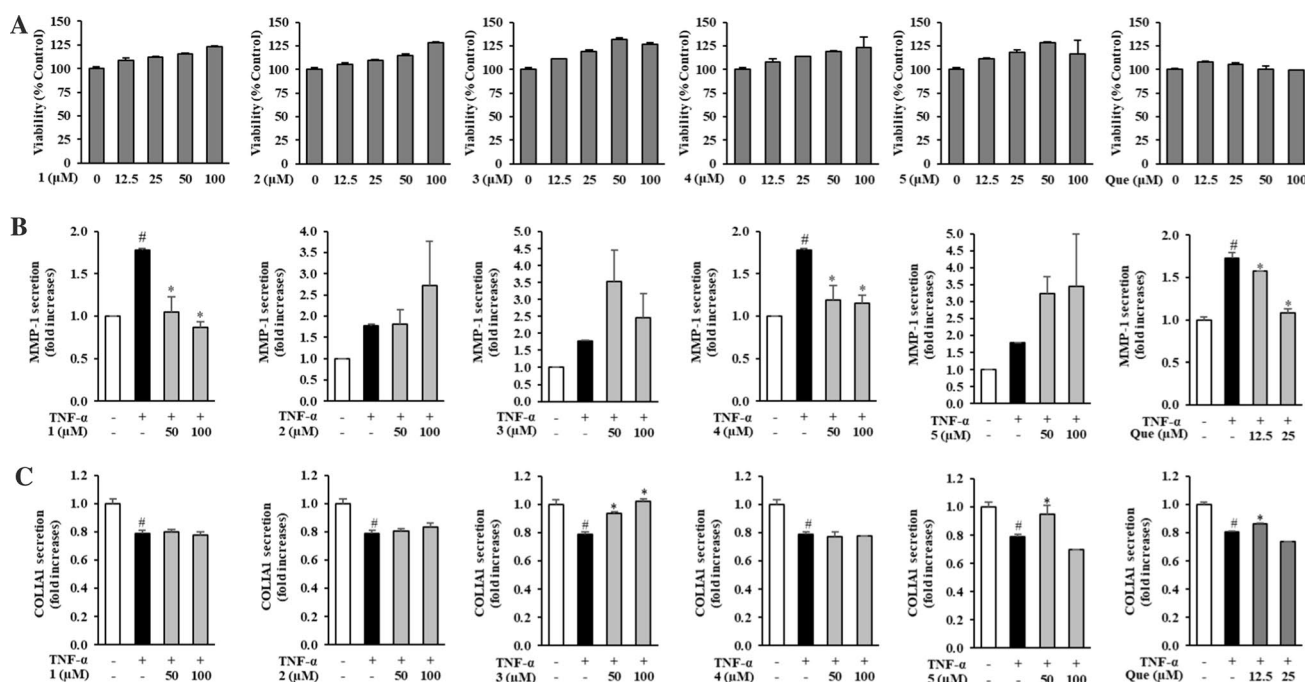
Furthermore, in this study, the ginsenoside content in TG2 and TG3 was generally lower than that reported in a previous study.

Ginsenosides have been reported to be the most important biological component of ginseng. Ginsenoside Rf (**2**) has anti-nociceptive and anti-inflammatory properties, while ginsenoside Re (**4**) and ginsenoside Rd (**5**) exhibit anti-diabetic and neuroprotective properties, respectively [41–43]. Ginsenosides Rg1 (**1**) and Rb1 (**3**), considered to be the two primary ginsenosides [44], have some opposing pharmacological properties. Ginsenoside Rg1 (**1**) stimulates the central nervous system, inhibits lipid peroxidation, and produces hepatoprotection and neuroprotective effects [45–47], while

ginsenoside Rb1 (**3**) suppresses the central nervous system and inhibits lipid peroxidation [45].

We investigated the anti-skin-aging properties of ginsenosides Rg1 (**1**), Rf (**2**), Rb1 (**3**), Re (**4**), and Rd (**5**) of TG. First, the impact of these samples on the viability of HDFs was assessed. Ginsenosides Rg1 (**1**), Rf (**2**), Rb1 (**3**), Re (**4**), Rd (**5**), and Que were not cytotoxic to HDFs in the applied concentration range (Fig. 5A). Thus, we used concentrations of 50 and 100 μ M when testing the compounds in subsequent assays.

We evaluated the anti-skin-aging properties of these samples on TNF- α -stimulated HDFs using two markers: MMP-1 and COLIA1. The TNF- α -induced increase in MMP-1

**Fig. 5** Effect of ginsenosides Rg1 (**1**), Rf (**2**), Rb1 (**3**), Re (**4**), and Rd (**5**) of TG and Que on cell viability (A), MMP-1 (B) and COLIA1 secretion (C) of HDFs. The viability of cells was assessed using EZ-Cytox kit. The content of MMP-1 and COLIA1 in the superna-

tant was determined using an ELISA kit. Data are presented as the mean $\pm$ SD (*n* = 3). #*p* < 0.05 vs. non-treated cells, **p* < 0.05 vs. TNF- α -treated cells

secretion was decreased in groups exposed to ginsenoside Rg1 (1), ginsenoside Re (4), and Que in a dose-dependent manner (Fig. 5B). Ginsenosides Rf (2), Rb1 (3), and Rd (5) did not attenuate MMP-1 secretion. Furthermore, treatment with 20 ng/mL TNF- α significantly reduced COLIA1 secretion but this was restored, in a dose-dependent manner, by ginsenoside Rb1 (3). At a concentration of 50 μ M, ginsenoside Rd (5) also significantly enhanced COLIA1 secretion, while ginsenosides Rg1 (1), Rf (2), and Re (4) did not reverse the reduction in COLIA1 secretion induced by TNF- α in HDFs. 12.5 μ M Que (positive control) resulted in a significant recovery of COLIA1 secretion (Fig. 5C). Based on these data, ginsenosides Rg1 (1), Rb1 (3), Re (4), and Rd (5) exhibited the potential to prevent skin-aging induced by TNF- α in HDFs via the inhibition of MMP-1 secretion and increase in COLIA1 secretion.

Conclusion

Our results have demonstrated the anti-skin-aging effects of TG and its major ginsenosides in TNF- α -stimulated HDFs by attenuating MMP-1 secretion and increasing COLIA1 secretion. Thus, TG and its major ginsenosides are potential agents for preventing skin aging. Because of its effectiveness in determining and quantifying ginsenosides Rg1 (1), Rf (2), Rb1 (3), Re (4), and Rd (5) in TG, we conclude that HPLC/ELSD is a useful tool and that supercritical fluid CO₂ extraction is optimal for yielding ginsenosides and developing health supplements or drugs.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11418-022-01633-2>.

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References

- Ganceviciene R, Liakou AI, Theodoridis A, Makrantonaki E, Zouboulis CC (2012) Skin anti aging strateg Dermatendocrinol 4:308–319. <https://doi.org/10.4161/derm.22804>
- Zhang S, Duan E (2018) Fighting against skin aging: the way from bench to bedside. Cell Transpl 27:729–738. <https://doi.org/10.1177/0963689717725755>
- Fisher GJ, Voorhees JJ (1998) Molecular mechanisms of photo-aging and its prevention by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin in vivo. J Investig Dermatol Symp Proc 3:61–68. <https://doi.org/10.1038/jidsymp.1998.15>
- Poljšak B, Dahmane RG, Godić A (2012) Intrinsic skin aging: the role of oxidative stress. Acta Dermatovenol Alp Panon Adriat 21:33–36. <https://doi.org/10.2478/v10162-012-0012-5>
- Borg M, Brincat S, Camilleri G, Schembri-Wismayer P, Brincat M, Calleja-Aguis J (2013) The role of cytokines in skin aging. Climacteric 16:514–521. <https://doi.org/10.3109/13697137.2013.802303>
- Lee S, Yu JS, Phung HM, Lee JG, Kim KH, Kang KS (2020) Potential anti-skin aging effect of (-)-catechin isolated from the root bark of *Ulmus davidiana* var japonica in tumor necrosis factor- α -stimulated normal human dermal fibroblasts. Antioxidants 9(10):981. <https://doi.org/10.3390/antiox9100981>
- Lee S, Nguyen QN, Phung HM, Shim SH, Kim D, Hwang GS, Kang KS (2021) Preventive effects of anthraquinones isolated from an Endophytic fungus, Colletotrichum sp JS-0367 in tumor necrosis factor- α -stimulated damage of human dermal fibroblasts. Antioxidants 10(2):200. <https://doi.org/10.3390/antiox10020200>
- Parish LC, Crissey JT (1988) Cosmetics: a historical review. Clin Dermatol 6:1–4. [https://doi.org/10.1016/0738-081X\(88\)90024-7](https://doi.org/10.1016/0738-081X(88)90024-7)
- Jang H-Y, Park H-S, Kwon K-R, Rhim T-J (2008) A study on the comparison of antioxidant effects among wild ginseng, cultivated wild ginseng, and cultivated ginseng extracts. J Pharmacopuncture 11:67–78. <https://doi.org/10.3831/KPI.2008.11.3.067>
- Cho H-C, Lee S-G, Kwon K-R (2004) An experimental study on apoptosis of cultivated wild ginseng distilled herbal acupuncture by concentration level. J Pharmacopuncture 7:5–17. <https://doi.org/10.3831/KPI.2004.7.2.005>
- Lee YE, Lee HJ, Kim JS (2015) The anti-wrinkle effects of cultivated wild ginseng pharmacopuncture. J Acupunct Res 32(2):97–103
- Kim H-R, Lee C-H, Jung MY, Kim J-S, Kim H-J, Jeon H-S, Lee S-H, Kim J-H, Shin M-J, Ma S-Y (2019) Anti-obesity effects of cultivated ginseng, wild simulated ginseng and red ginseng extracts. Herb Formula Sci 27:269–284
- Seo H-J, Eo HJ, Kim HJ, Jeon KS, Park GH, Hong SC, Jeong JB (2020) Effects of cultivated wild panax ginseng extract on the proliferation, differentiation and mineralization of osteoblastic MC3T3-E1 cells. Korean J Plant Res 33:227–236. <https://doi.org/10.7732/kjpr.2020.33.4.227>
- Liang Y, Zhao S (2008) Progress in understanding of ginsenoside biosynthesis. Plant Biol 10:415–421. <https://doi.org/10.1111/j.1438-8677.2008.00064.x>
- Lee DG, Lee J, Yang S, Kim K-T, Lee S (2015) Identification of dammarane-type triterpenoid saponins from the root of *Panax ginseng*. Nat Prod Sci 21:111–121
- Christensen LP (2008) Ginsenosides: chemistry, biosynthesis, analysis, and potential health effects. Adv Food Nutr Res 55:1–99. [https://doi.org/10.1016/S1043-4526\(08\)00401-4](https://doi.org/10.1016/S1043-4526(08)00401-4)
- Shibata S (1982) Chemistry of components in ginseng. J Tradit Sino Jpn Med 3:62–69
- Liu L, Huang J, Hu X, Li K, Sun C (2011) Simultaneous determination of ginsenoside (G-Re, G-Rg1, G-Rg2, G-F1, G-Rh1) and protopanaxatriol in human plasma and urine by LC–MS/MS and its application in a pharmacokinetics study of G-Re in volunteers. J Chromatogr B 879:2011–2017. <https://doi.org/10.1016/j.jchromb.2011.05.018>

19. Yamabe N, Kim Y-J, Lee S, Cho E-J, Park S-H, Ham J, Kim HY, Kang KS (2013) Increase in antioxidant and anticancer effects of ginsenoside Re–lysine mixture by Maillard reaction. *Food Chem* 138:876–883. <https://doi.org/10.1016/j.foodchem.2012.12.004>
20. Duan Z, Deng J, Dong Y, Zhu C, Li W, Fan D (2017) Anticancer effects of ginsenoside Rk3 on non-small cell lung cancer cells: in vitro and in vivo. *Food Funct* 8:3723–3736. <https://doi.org/10.1039/C7FO00385D>
21. Lu J-M, Yao Q, Chen C (2009) Ginseng compounds: an update on their molecular mechanisms and medical applications. *Curr Vasc Pharmacol* 7:293–302. <https://doi.org/10.2174/157016109788340767>
22. Wu W, Jiao C, Li H, Ma Y, Jiao L, Liu S (2018) LC-MS based metabolic and metabonomic studies of *Panax ginseng*. *Phytochem Anal* 29:331–340. <https://doi.org/10.1002/pca.2752>
23. Son TK, Eguchi T, Shoyama Y, Tanaka H (2019) ELISA for the detection of marker compound for crop fertilizer use of various medicinal crop extracts using bacterium. *J Fac Agric Kyushu Univ* 64:27–32. <https://doi.org/10.5109/2231630>
24. Lloyd G, McCown B (1980) Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Comb Pro Int Plant Propag Soc* 30:421–427
25. Owen HR, Miller AR (1982) An examination and correction of plant tissue culture basal medium formulations plant Cell. *Tissue Organ Cult* 28:147–150. <https://doi.org/10.1007/BF00055509>
26. Kitts DD, Wijewickreme AN, Hu C (2000) Antioxidant properties of a North American ginseng extract. *Mol Cell Biochem* 203:1–10. <https://doi.org/10.1023/A:1007078414639>
27. Shibata S (2001) Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. *J Korean Med Sci* 16(Suppl):S28–S37. <https://doi.org/10.3346/jkms.2001.16.S.S28>
28. Attele AS, Zhou Y-P, Xie J-T, Wu JA, Zhang L, Dey L, Pugh W, Rue PA, Polonsky KS, Yuan C-S (2002) Antidiabetic effects of *Panax ginseng* berry extract and the identification of an effective component. *Diabetes* 51:1851–1858. <https://doi.org/10.2337/diabetes.51.6.1851>
29. Siddiqi MH, Siddiqi MZ, Ahn S, Kang S, Kim Y-J, Sathishkumar N, Yang D-U, Yang D-C (2013) Ginseng saponins and the treatment of osteoporosis: mini literature review. *J Ginseng Res* 37:261–268. <https://doi.org/10.5142/jgr.2013.37.261>
30. Kim JH, Yi Y-S, Kim M-Y, Cho JY (2017) Role of ginsenosides, the main active components of *Panax ginseng*, in inflammatory responses and diseases. *J Ginseng Res* 41:435–443. <https://doi.org/10.1016/j.jgr.2016.08.004>
31. Dunaway S, Odin R, Zhou L, Ji L, Zhang Y, Kadekaro AL (2018) Natural antioxidants: multiple mechanisms to protect skin from solar radiation. *Front Pharmacol* 9:392. <https://doi.org/10.3389/fphar.2018.00392>
32. Kim E, Kim D, Yoo S, Hong YH, Han SYm Jeong S, Jeong D, Kim J-H, Cho JY, Park J, (2018) The skin protective effects of compound K, a metabolite of ginsenoside Rb1 from *Panax ginseng*. *J Ginseng Res* 42:218–224. <https://doi.org/10.1016/j.jgr.2017.03.007>
33. Song KC, Chang T-S, Lee H, Kim J, Park JH, Hwang GS (2012) Processed *Panax ginseng*, sun ginseng increases type I collagen by regulating MMP-1 and TIMP-1 expression in human dermal fibroblasts. *J Ginseng Res* 36:61–67. <https://doi.org/10.5142/jgr.2012.36.1.61>
34. Lim H, Kim HP (2007) Inhibition of mammalian collagenase, matrix metalloproteinase-1, by naturally-occurring flavonoids. *Planta Med* 73:1267–1274. <https://doi.org/10.1055/s-2007-990220>
35. Lang Q, Wai CM (2001) Supercritical fluid extraction in herbal and natural product studies—a practical review. *Talanta* 53:771–782. [https://doi.org/10.1016/S0039-9140\(00\)00557-9](https://doi.org/10.1016/S0039-9140(00)00557-9)
36. Kim I-W, Hong H-D, Choi SY, Hwang D-H, Her Y, Kim S-K (2011) Characterizing a full spectrum of physico-chemical properties of ginsenosides Rb1 and Rg1 to be proposed as standard reference materials. *J Ginseng Res* 35:487–496. <https://doi.org/10.5142/jgr.2011.35.4.487>
37. Kim SN, Ha YW, Shin H, Son SH, Wu SJ, Kim YS (2007) Simultaneous quantification of 14 ginsenosides in *Panax ginseng* CA Meyer (Korean red ginseng) by HPLC-ELSD and its application to quality control. *J Pharm Biomed* 45:164–170. <https://doi.org/10.1016/j.jpba.2007.05.001>
38. Hong HD, Choi SY, Kim YC, Lee YC, Cho CW (2009) Rapid determination of ginsenosides Rb1, Rf, and Rg1 in Korean ginseng using HPLC. *J Ginseng Res* 33:8–12. <https://doi.org/10.5142/JGR.2009.33.1.008>
39. Lee DG, Lee JS, Kim K-T, Kim HY, Lee S (2019) Analysis of major ginsenosides in various ginseng samples. *J Appl Biol Chem* 62:87–91. <https://doi.org/10.3839/jabc.2019.013>
40. Ok S, Kang JS, Kim KM (2016) Simultaneous analysis method for polar and non-polar ginsenosides in cultivated wild ginseng by reversed-phase HPLC-CAD. *J Life Sci* 26:247–252. <https://doi.org/10.5352/JLS.2016.26.2.247>
41. Xie J-T, Mehendale SR, Li X, Quigg R, Wang X, Wang C-Z, Wu JA, Aung HH, Rue PA, Bell GI (2005) Anti-diabetic effect of ginsenoside Re in ob/ob mice. *Biochim Biophys Acta Mol Basis Dis* 1740:319–325. <https://doi.org/10.1016/j.bbadis.2004.10.010>
42. Cong L, Chen W (2016) Neuroprotective effect of ginsenoside Rd in spinal cord injury rats. *Basic Clin Pharmacol Toxicol* 119:193–201. <https://doi.org/10.1111/bcpt.12562>
43. Kim MK, Kang H, Baek CW, Jung YH, Woo YC, Choi GJ, Shin HY, Kim KS (2018) Antinociceptive and anti-inflammatory effects of ginsenoside Rf in a rat model of incisional pain. *J Ginseng Res* 42:183–191. <https://doi.org/10.1016/j.jgr.2017.02.005>
44. Oura H, Hiai S, Odaka Y, Yokozawa T (1975) Studies on the biochemical action of ginseng saponin: I. purification from ginseng extract of the active component stimulating serum protein biosynthesis. *J Biochem* 77:1057–1065. <https://doi.org/10.1093/oxfordjournals.jbchem.a130806>
45. Deng H-I, Zhang J-t (1991) Anti-lipid peroxidative effect of ginsenoside Rb1 and Rg1. *Chin Med J* 104:395–398. <https://doi.org/10.5555/cmj.0366-6999.104.05.p395.01>
46. Rui W, Yan-Nan L, Guang-Ji W, Hai-Ping H, Xiao-Lan W, Fang Z (2009) Neuroprotective effects and brain transport of ginsenoside Rg1. *Chin J Nat Med* 7:315–320. [https://doi.org/10.1016/S1875-5364\(10\)60024-6](https://doi.org/10.1016/S1875-5364(10)60024-6)
47. Gao Y, Chu S, Zhang Z, Chen N (2017) Hepatoprotective effects of ginsenoside Rg1—A review. *J Ethnopharmacol* 206:178–183. <https://doi.org/10.1016/j.jep.2017.04.012>

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