



Research Article

Optimization of the extraction process of high levels of chlorogenic acid and ginsenosides from short-term hydroponic-cultured ginseng and evaluation of the extract for the prevention of atopic dermatitis



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ABSTRACT

Background: Short-term hydroponic-cultured ginseng (sHCG), which is 1-year-old ginseng seedlings cultivated for 4 weeks in a hydroponic system, is a functional food item with several biological effects. However, the optimal extraction conditions for sHCG, and the bioactivity of its extracts, have not been evaluated.

Methods: Chlorogenic acid (CGA) and ginsenoside contents were evaluated in sHCG, white ginseng (WG), and red ginseng (RG) using high-performance liquid chromatography. Response surface methodology (RSM) was used to optimize the extraction conditions (temperature and ethanol concentration) to maximize the yield of dry matter, CGA, and four ginsenosides (Re, Rg₁, Rb₁, and Rd) from sHCG. The optimal extraction conditions were applied to pilot-scale production of sHCG extracts. The expression levels of tumor necrosis factor (TNF)- α /interferon (IFN)- γ -induced thymic and activation-regulated chemokines (TARC/CCL17) were measured after treatment with sHCG, WG, and RG extracts, and the effects of their bioactive compounds (CGA and four ginsenosides) on human skin keratinocytes (HaCaTs) were evaluated.

Results: CGA and four ginsenosides, which are bioactive compounds of sHCG, significantly inhibited TNF- α /IFN- γ -induced TARC/CCL17 expression. The optimal sHCG extraction conditions predicted by the RSM models were 80 °C and 60% ethanol (v/v). The sHCG extracts produced at the pilot scale under optimal conditions greatly alleviated TNF- α /IFN- γ -induced TARC/CCL17 production compared with WG and RG extracts.

Conclusions: Pesticide-free sHCG extracts, which contain high levels of CGA and the ginsenosides Re, Rg₁, Rb₁, and Rd as bioactive compounds, may have therapeutic potential for atopic diseases.

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1. Introduction

The root of *Panax ginseng* Meyer (Korean ginseng) has been used as traditional medicine in East Asian countries for more than 2000 years [1]. Various processed products from *P. ginseng* have been introduced globally. *Panax ginseng* has antioxidant [2] and anti-inflammatory [3] properties; thus, it is under investigation for its therapeutic effects on skin disorders, including atopic dermatitis (AD) [4]. Intake of red ginseng (RG) extract reportedly attenuated eczema, transepidermal water loss (TEWL), and skin squamation in patients with AD [5,6]. Also, an RG extract decreased 1-fluoro-2,4-

dinitrobenzene-induced ear thickness, TEWL, and levels of immunoglobulin E, thymic and activation-regulated chemokines (TARC/CCL17), thymic stromal lymphopoietin, and tumor necrosis factor (TNF)- α in mice [7–9].

The beneficial effects of *P. ginseng* are attributable to ginsenosides, which are the main active compounds in its roots [10]. However, phenolic compounds, including phenolic acids and flavonoids, have also been detected in the fruits, leaves, and roots of *P. ginseng* aged 3–6 years. Higher antioxidant activity has been measured in ginseng leaves and fruits than in roots, possibly in association with the 4–9-fold higher phenolic contents of fruits and leaves than roots [11]. However, the beneficial effects of these phenolic compound-rich fruits and leaves remain unclear.

Short-term hydroponic-cultured ginseng (sHCG) [12] is a widely available, eco-friendly plant that cultivated in pesticide-free indoor farms. Unlike typically grown ginseng, which is usually sold in the form of dried roots, sHCG is harvested as a whole plant, consisting of roots, stems, and leaves. Ginseng roots contain high levels of ginsenosides, whereas the stems and leaves contain high levels of phenolic compounds; therefore, sHCG has a nutritional advantage over ginseng because all parts of the plant are used. Our previous study reported that sHCG, in the form of 1-year-old ginseng seedlings grown hydroponically for 21 days, exhibited higher antioxidant activity than 5-year-old ginseng plants [13]. Contents of total ginsenosides and four ginsenosides (Re, Rg₁, Rb₁, and Rd) were significantly higher in sHCG than in 5-year-old ginseng. Additionally, sHCG has an almost fourfold higher total phenolic content compared to 5-year-old ginseng. However, the beneficial physiological effects of sHCG are not clear.

Several variable factors determine the extractability of saponin, such as temperature, solvents, extraction time, and the liquid to solid ratio. However, it is difficult to determine which factor is most important due to their complex interactions. Therefore, response surface methodology (RSM) has been used to determine optimal extraction conditions in studies attempting to maximize the yield of saponins from medicinal plants [14–16] and Rg₁ and phenolics from RG [11].

However, to date, no study has evaluated the extraction conditions for sHCG and the bioactivity of the extract. In this study, the optimal extraction conditions for sHCG were evaluated using RSM modeling, with the aim of maximizing the yields of bioactive compounds abundant in sHCG but present at lower levels in white ginseng (WG) and RG. Additionally, pilot-scale sHCG extracts were produced using the optimal extraction conditions for future commercial production and the effects of the extracts on TNF- α /IFN- γ -induced TARC levels in human keratinocytes were evaluated as indicators of AD disease severity [17].

2. Materials and methods

2.1. Preparation of sHCG

sHCG was grown in an agricultural corporation, Farmcraft (Gimpo, Korea), as previously described [13] and harvested on June 8, 2018. Briefly, ginseng seedling roots that had been cultivated in soil for 1 year were transplanted into a hydroponic system and incubated for 28 days at 22 °C under a light-emitting diode (LED) lighting system with a red:blue ratio of 5:2 at a photon flux density of 100 $\mu\text{mol}/\text{m}^2/\text{s}$. Silicate mineral water (pH 6.5–7.5) was sprayed twice daily. The 5-year-old WG (commonly cultured form) was obtained from the Anseong Ginseng National Agricultural Cooperative Federation (Anseong, Korea) and RG was from the Korea Ginseng Corporation (Seoul, Korea). The ginseng samples were washed with distilled water and stored at –70 °C until required.

2.2. Standards and reagents

Standards for the ginsenosides Re, Rg₁, Rb₁, and Rd were purchased from ChemFaces Biochemical Co. (Wuhan, China). The chlorogenic acid (CGA) standard was obtained from Sigma-Aldrich (St. Louis, MO). The organic solvents used in this study were of ultrapure grade.

2.3. Extraction of sHCG

sHCG was lyophilized and ground to a particle size of <500 μm . The powder was stored at –80 °C. Frozen sHCG was thawed at room temperature immediately before extraction. The extraction yields of Rg₁ and phenolics are mainly determined by the ethanol ratio and temperature, not extraction time [11]; Thus, when evaluating extraction efficiency, only the ethanol ratio (0–70% v/v) and temperature (50–80 °C) were considered. The ethanol ratio was adjusted over a wide range because ginsenoside hydrophobicity varies by the number of sugar moieties and CGA is hydrophilic; the temperature range of 50–80 °C was selected based on previous studies on standardization of ginseng processing [18]. Because ethanol can evaporate near the boiling point during heat treatment, the extraction chamber was connected to a cooling condenser to maintain the ethanol concentration. The solid-to-liquid ratio was maintained at 50 g/L for all extraction batches, and the extraction time was 6 h; these conditions are commonly used for pilot-scale production. The extracted solution was passed through Advantec No. 1 filter paper (6- μm pore size, Advantec Co., Ltd, Tokyo, Japan), the filtrate was concentrated, and residual ethanol was removed in a vacuum rotary evaporator. The concentrate was lyophilized, and the resulting powder, the sHCG extract, was stored at –80 °C for chromatography.

Pilot-scale production was carried out using a sequential extraction method selected to maximize yields. Successive extractions were performed at 80 °C with 60% ethanol, 70 °C with 30% ethanol, and 60 °C with 0% ethanol. These conditions were also applied for the extraction of 5-year-old ginseng.

2.4. Determination of ginsenoside and CGA contents

Four ginsenosides (Re, Rg₁, Rb₁, and Rd) and CGA were analyzed using high-performance liquid chromatography with diode array detection as described previously [13]. Briefly, 1 g of sample was extracted with 10 mL of 70% (v/v) aqueous methanol for 2 h to analyze ginsenosides, and with 10 mL of 80% (v/v) aqueous methanol for 24 h to analyze CGA. The extracted solution was centrifuged (850 \times g, 10 min, 4 °C), filtered through a 0.22- μm nylon membrane, and analyzed with an Ultimate 3000 HPLC system (Dionex, Thermo Fisher Scientific, Waltham, MA) using Inno C-18 column (4.6 \times 250 mm, 5 μm ; Young Jin Biochrom, Seongnam, Korea). The column temperature was maintained at 30 °C. Solvent A was 0.3% trifluoroacetic acid, and solvent B was acetonitrile. The four ginsenosides and CGA were detected at 215 and 340 nm, respectively. Representative HPLC chromatograms are shown in Fig. 1; these reveal the peaks of the four ginsenoside standards (Fig. 1A); the CGA standard peak (Fig. 1B); the Re, Rg₁, Rb₁, and Rd peaks in the sHCG extract (Fig. 1C); and the CGA peak in the sHCG extract (Fig. 1D). The levels of the four ginsenosides in ginseng were quantified using an external standard curve ($R^2 > 0.99$).

2.5. Experimental design and RSM modeling

To assess the relationships of temperature and ethanol concentration with extraction efficiency, RSM was conducted with a central composite design (CCD) using Design-Expert software (Stat-

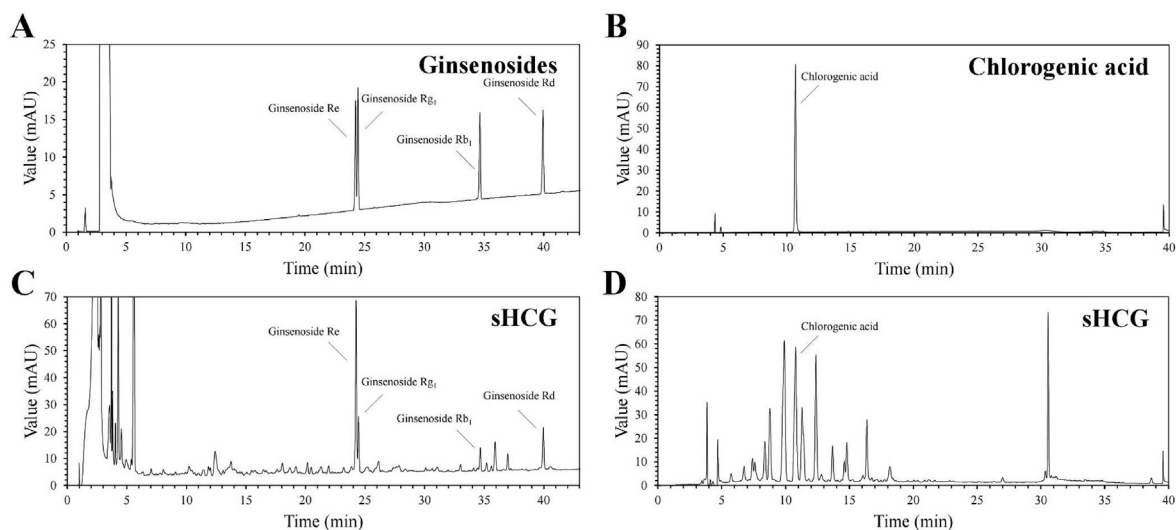


Fig. 1. Representative high-performance liquid chromatography (HPLC) chromatograms showing the peaks of four ginsenosides (Re, Rg₁, Rb₁, and Rd) and chlorogenic acid (CGA). Ginsenosides and CGA were detected using a diode array operating at 215 nm and 340 nm respectively. (A) The peaks of a mixture of the four ginsenoside standards; (B) the peak of the CGA standard; (C) four ginsenoside (Re, Rg₁, Rb₁, and Rd) peaks in the short-term hydroponic-cultured ginseng (sHCG) extract; and, (D) the CGA peak in the sHCG extract.

Ease Inc., Minneapolis, MN). The two independent variables were temperature (X_1 ; 50–80 °C) and ethanol concentration (X_2 ; 0–70% v/v), and the dependent variables were dry matter (Y_1 ; %, w/w), CGA (Y_2 ; mg/g), Re (Y_3 ; mg/g), Rg₁ (Y_4 ; mg/g), Rb₁ (Y_5 ; mg/g), and Rd (Y_6 ; mg/g) yields. Data were obtained by running the extraction process 29 times with different values set for the independent variables, according to the CCD. Based on the results, the RSM model was fitted to the following second-order polynomial quadratic equation (Equation [1]):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{i < j} \beta_{ij} X_i X_j \quad [1]$$

Where Y is a dependent variable representing the extraction response, X_i and X_j are the independent variables, and β_i , β_{ii} , and β_{ij} are regression coefficients representing linear, quadratic, and interaction terms, respectively.

2.6. Measurement of TARC/CCL17 levels

Human skin keratinocytes (HaCaTs) were purchased from CLS Cell Lines Service GmbH (Eppelheim, Germany). The HaCaTs were maintained at 37 °C in an atmosphere of 5% CO₂ in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin (100,000 units/L)/streptomycin (100 mg/L).

To evaluate the effect of the extracts on TARC/CCL17 production, HaCaTs were seeded at a density of 2×10^5 cells/well in six-well plates for sandwich enzyme-linked immunosorbent assay (ELISA). The HaCaTs were pretreated with extracts of sHCG, WG, or RG for 1 h and stimulated with 10 ng/mL TNF- α /IFN- γ for 24 h. The TARC/CCL17 levels in culture media were measured using an ELISA kit (R&D Systems, Minneapolis, MN) following the manufacturer's instructions. Cell viability was assessed by MTT assay [19].

2.7. Statistical analysis

Data are expressed as the means \pm standard deviations. The significance of differences among groups was assessed using one-way ANOVA with Duncan test ($P < 0.05$ or $P < 0.01$). Statistical

analysis was carried out using SPSS version 23.0 for Windows (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Inhibitory effects of bioactive sHCG compounds on TNF- α /IFN- γ -induced TARC/CCL17 production in HaCaT cells

Fifty-one phenolic compounds were extracted from sHCG, WG, and RG, and analyzed using ultra-high-performance liquid chromatography-tandem mass spectrometry by reference to a database of mass-fragmentation patterns. The CGA content was markedly higher in sHCG than in WG and RG. HPLC-UV quantification revealed a CGA content of 12.7 mg/100 g dry sHCG, which was 2.5- and 13.3-fold higher than the contents in WG and RG, respectively (Fig. 2A). Similarly, Chung et al. reported that of 23 phenolic compounds, CGA and p- and m-coumaric acids were the major phenolic compounds detected, particularly in leaves of ginseng aged 3–6 years [11]. Because only roots of WG and RG were analyzed, the higher content of CGA in sHCG may be attributed to the leaves. CGA is biosynthesized and stored during the early stages of leaf development in other plants, such as coffee trees and perforate St John's wort [20,21]. Therefore, it is reasonable to assume that the ginsenoside and CGA contents are higher in sHCG containing leaves and stems cultured in the early growth period than in conventionally grown ginseng and RG. In addition to CGA, 22 ginsenosides of sHCG were profiled in our previous study [13], and 4 ginsenosides (Re, Rg₁, Rb₁ and Rd) were selected as representative bioactive compounds. These four ginsenosides and CGA are known to be anti-inflammatory, antioxidant and immune-enhancing agents [22–26].

AD is a chronic inflammatory skin disease caused by impaired immune regulation, genetic abnormalities, and hypersensitivity to environmental factors such as allergens [27,28]. Keratinocytes are epidermal cells that play a critical role in maintaining epidermal barrier function, thus protecting the body from allergens and pathogens [29]. When stimulated by immune triggers (e.g., allergens or cytokines produced by T cells such as TNF- α and IFN- γ), keratinocytes release inflammatory mediators (e.g., TARC/CCL17) and macrophage-derived chemokines [30]. TARC/CCL17 and the

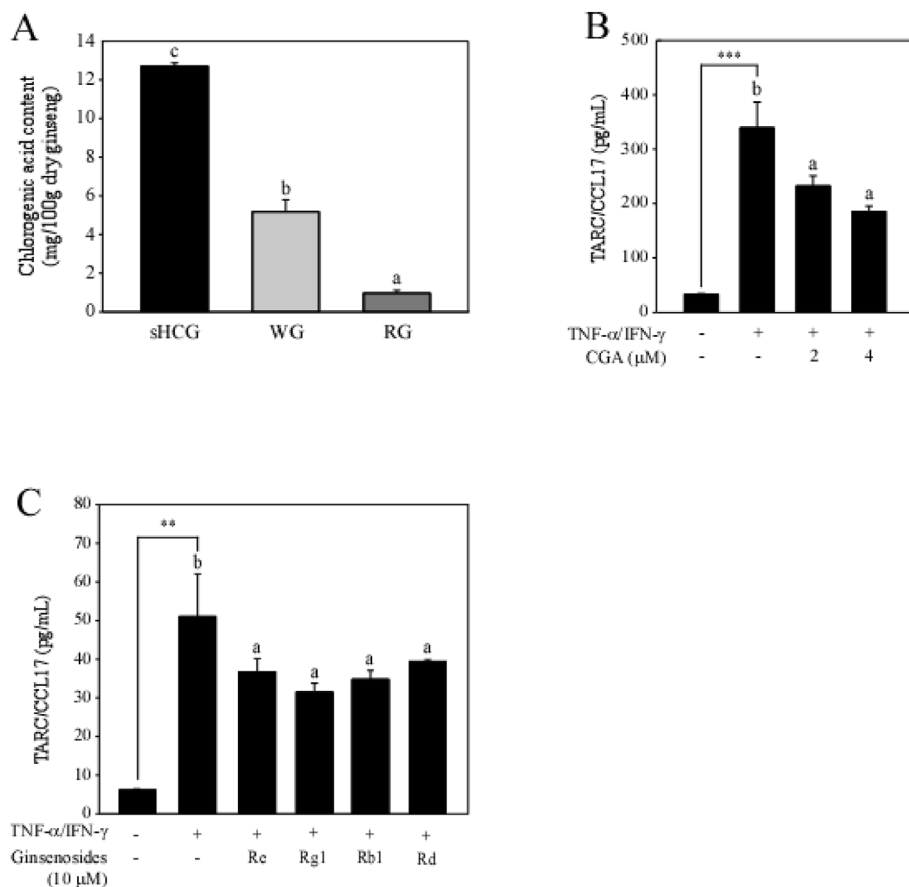


Fig. 2. Comparison of CGA contents among ginseng sources, and the inhibitory effects of bioactive compounds in sHCG on the production of the atopic dermatitis (AD)-associated chemokine TARC/CCL17 in human skin keratinocytes (HaCaTs). (A) CGA content (mg/100 g dry ginseng) in sHCG, white ginseng (WG), and red ginseng (RG). (B) The effects of CGA treatment (2 and 4 μM) on tumor necrosis factor (TNF)-α/interferon (IFN)-γ-induced TARC/CCL17 production in HaCaTs. (C) The effects of treatment with 10 μM of ginsenoside (Re, Rg₁, Rb₁, or Rd) on TNF-α/IFN-γ-induced TARC/CCL17 production in HaCaTs. Asterisks indicate significant differences from the control (***P* < 0.01; ****P* < 0.001). Different lowercase letters above the bars indicate significant differences between groups (*P* < 0.05).

chemokines promote penetration of inflamed tissue by T cells [31]. In particular, TARC/CCL17, which has high affinity for CCR4+ T cells, is found only in AD lesions; higher levels are associated with more severe AD [32,33].

To examine the AD-preventing effects of CGA and the four ginsenosides, it was evaluated whether secretion of TNF-α/IFN-γ-induced TARC/CCL17 was decreased by the treatment of HaCaT cells with CGA and the four ginsenosides. Treatment of HaCaTs with 2 and 4 μM CGA significantly reduced TNF-α/IFN-γ-induced production of TARC/CCL17 by 32% and 45%, respectively (Fig. 2B). Also, Re, Rg₁, Rb₁, and Rd at 10 μM significantly reduced TNF-α/IFN-γ-induced TARC/CCL17 production in HaCaTs by 28%, 38%, 31%, and 22%, respectively (Fig. 2C).

3.2. Optimization of sHCG extraction conditions to maximize CGA and ginsenoside yields

High concentrations of CGA (Fig. 2A), Re, Rg₁, Rb₁, and Rd can be found in sHCG [13]. CGA and the four ginsenosides inhibited TNF-α/IFN-γ-induced TARC/CCL17 production in HaCaTs (Fig. 2B and C). Therefore, it was hypothesized that sHCG extracts with high concentrations of these compounds can be used in the prevention and treatment of AD. Therefore, RSM modeling was used to determine the optimal temperature (*X*₁) and ethanol concentration (*X*₂) for extracting these compounds from sHCG.

RSM enables the identification of optimal conditions from predictive models. In industry, RSM is applied to increase productivity, reduce costs, and control processes. RSM has been used to optimize the extraction of bioactive compounds from *Panax* plants, including polysaccharides from WG (*P. ginseng*) [34], Rg₁ and phenolics from RG (*P. ginseng*) [11], polysaccharides from *Panax japonicus* Meyer [35], and flavonoids from *Panax notoginseng* stems and leaves [36]. However, this is the first RSM study of the optimal extraction conditions for sHCG for maximizing the yields of CGA and ginsenosides.

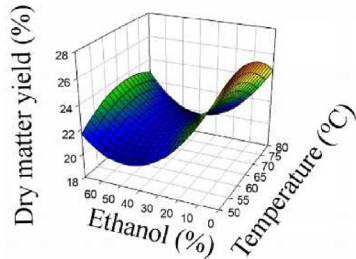
3.2.1. Experimental data

Twenty-nine runs were conducted to assess the effects of temperature (*X*₁: 50 °C, 65 °C, and 80 °C) and ethanol concentration (*X*₂: 0%, 35%, and 70%) on the yields of bioactive compounds from sHCG. The factors and responses of interest are listed in Table 1. The responses were dry matter (*Y*₁), CGA (*Y*₂), Re (*Y*₃), Rg₁ (*Y*₄), Rb₁ (*Y*₅), and Rd (*Y*₆) yields.

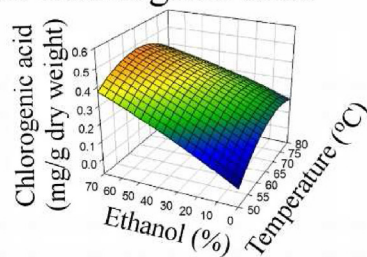
The dry matter yield (*Y*₁) ranged from 16.9% to 27.4% (w/w). The lowest dry matter yield was obtained on run 11 (*X*₁: 50 °C, *X*₂: 70%), and the highest on run 25 (*X*₁: 65 °C, *X*₂: 0%). The CGA (*Y*₂) yield ranged from 0.01 to 0.44 mg/g dry extract. The lowest CGA yield was observed on runs 12 (*X*₁: 50 °C, *X*₂: 0%), 17 (*X*₁: 50 °C, *X*₂: 0%), and 21 (*X*₁: 50 °C, *X*₂: 0%), and the highest on run 23 (*X*₁: 65 °C, *X*₂: 70%). For all extracts, the Re yield (*Y*₃) was higher than those of the other ginsenosides (*Y*₄, *Y*₅, and *Y*₆). This is consistent with our

A. Response surface graphs

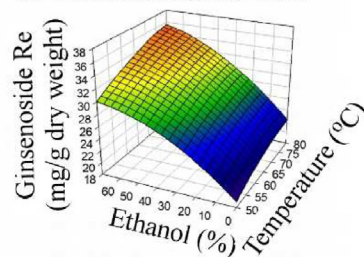
a. Dry matter yield



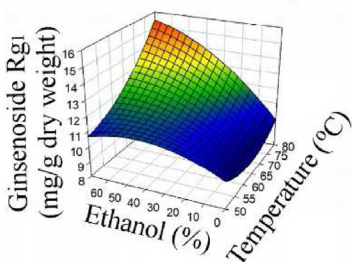
b. Chlorogenic acid



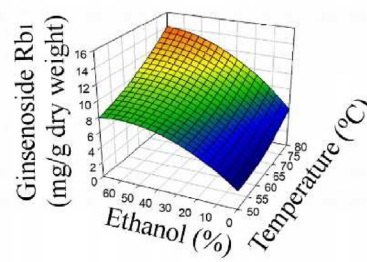
c. Ginsenoside Re



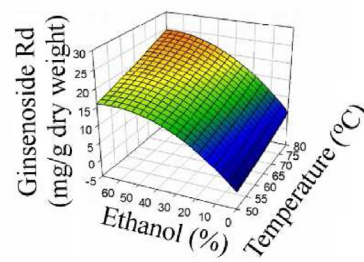
d. Ginsenoside Rg₁



e. Ginsenoside Rb₁

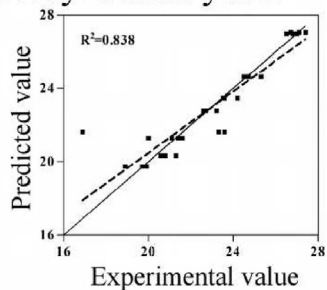


f. Ginsenoside Rd

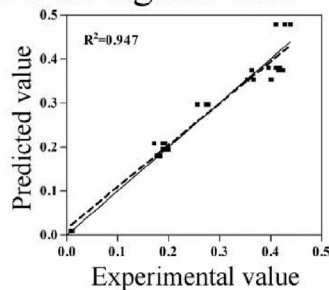


B. Plots of experimental and predicted values

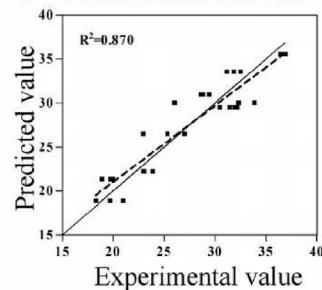
a. Dry matter yield



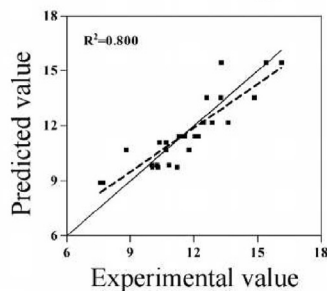
b. Chlorogenic acid



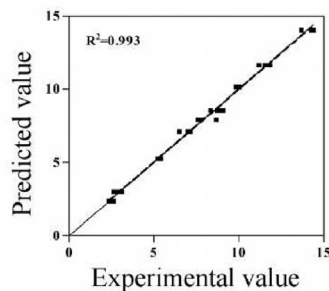
c. Ginsenoside Re



d. Ginsenoside Rg₁



e. Ginsenoside Rb₁



f. Ginsenoside Rd

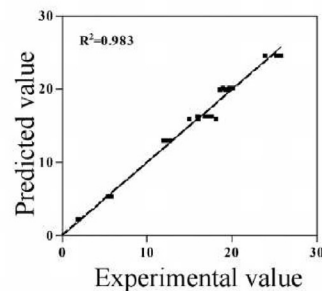


Fig. 3. The response surface model and statistical distribution of experimental and predicted values. (A) Three-dimensional plot of the regression model of temperature (50–80 °C) and ethanol concentration (0–70%) against (a) dry matter yield, (b) CGA, (c) Re, (d) Rg₁, (e) Rb₁, and (f) Rd. (B) Observed and predicted values of (a) dry matter yield, (b) CGA, (c) Re, (d) Rg₁, (e) Rb₁, and (f) Rd. Regression model accuracy was evaluated using the coefficient of determination (R^2).

Table 1
Data obtained using a central composite design matrix for optimization of dry-matter yield and the chlorogenic acid, Re, Rg₁, Rb₁, and Rd contents.

Run #	Factors		Responses					
	Temperature X ₁ (°C)	Ethanol concentration X ₂ (%)	Dry-matter yield Y ₁ (%)	Chlorogenic acid Y ₂ (mg/g)	Ginsenoside Re Y ₃ (mg/g)	Ginsenoside Rg ₁ Y ₄ (mg/g)	Ginsenoside Rb ₁ Y ₅ (mg/g)	Ginsenoside Rd Y ₆ (mg/g)
1	80 (+1)	0 (-1)	24.70	0.18	23.91	9.99	5.27	5.49
2	65 (0)	35 (0)	21.10	0.35	32.16	12.04	8.85	17.58
3	80 (+1)	0 (-1)	25.30	0.18	23.00	10.83	5.43	5.78
4	65 (0)	70 (+1)	23.60	0.41	31.19	12.43	10.01	18.61
5	80 (+1)	70 (+1)	23.20	0.42	36.86	16.12	14.39	25.75
6	65 (0)	35 (0)	21.40	0.40	31.47	11.49	8.73	16.77
7	50 (-1)	70 (+1)	23.60	0.36	26.05	11.76	7.60	14.94
8	80 (+1)	35 (0)	19.70	0.26	29.40	13.26	11.53	19.71
9	50 (-1)	35 (0)	20.80	0.19	25.35	10.71	7.00	12.38
10	65 (0)	70 (+1)	23.50	0.43	32.52	12.85	9.90	19.47
11	50 (-1)	70 (+1)	16.90	0.42	32.30	8.81	7.79	16.01
12	50 (-1)	0 (-1)	26.50	0.01	20.98	10.31	2.49	0.00
13	65 (0)	35 (0)	21.40	0.40	32.01	11.56	8.83	17.19
14	80 (+1)	0 (-1)	24.50	0.18	22.97	10.25	5.24	5.42
15	80 (+1)	70 (+1)	22.70	0.40	36.51	13.27	13.69	23.89
16	65 (0)	35 (0)	21.60	0.40	31.98	12.19	9.06	17.48
17	50 (-1)	0 (-1)	27.00	0.01	19.71	11.21	2.64	0.00
18	80 (+1)	35 (0)	18.90	0.28	28.78	14.83	11.82	20.10
19	50 (-1)	70 (+1)	23.30	0.42	33.86	10.69	8.68	18.11
20	65 (0)	35 (0)	20.00	0.37	30.49	11.29	8.33	15.95
21	50 (-1)	0 (-1)	26.80	0.01	18.27	10.03	2.32	0.00
22	80 (+1)	70 (+1)	22.60	0.41	36.40	15.38	14.26	25.21
23	65 (0)	70 (+1)	24.20	0.44	31.85	13.62	9.85	19.22
24	50 (-1)	35 (0)	21.30	0.19	26.99	10.69	7.14	12.75
25	65 (0)	0 (-1)	27.40	0.20	19.78	7.72	2.86	1.90
26	65 (0)	0 (-1)	26.70	0.19	18.93	7.58	2.64	1.84
27	50 (-1)	35 (0)	20.60	0.17	22.92	10.39	6.50	11.93
28	80 (+1)	35 (0)	19.90	0.27	28.58	12.59	11.18	18.94
29	65 (0)	0 (-1)	27.10	0.20	19.98	7.59	3.14	1.86

Coded factors (-1, 0, +1) corresponding to independent variables are shown in parentheses.

previous finding that the Re level in sHCG is two- to three-fold higher than those of Rd, Rb₁, and Rg₁ [13].

The contents of the four ginsenosides were as follows: Re, 18.27–36.86 mg/g dry extract; Rg₁, 7.58–16.12 mg/g dry extract; Rb₁, 2.32–14.39 mg/g dry extract; and Rd, 0–25.75 mg/g dry extract. The highest levels of ginsenosides were obtained on run 5, at the highest temperature and ethanol concentration (X₁: 80 °C, X₂: 70%).

3.2.2. RSM

Theoretical RSM runs were computed using the experimental data in Table 1. The experimental data were fitted to a second-order polynomial model (Equation [1]) to determine the regression model for each response. The resulting regression model contains only the coefficients significant at the 95% confidence level ($\alpha = 0.05$). The coefficients of the variables for each model and the statistical evaluations are presented in Supplementary Table 1.

The dry matter yield (Y₁) was not significantly affected by temperature (X₁) ($P > 0.05$), but increased as the ethanol concentration (X₂) became close to either extreme (X₂ = 0%, 70% v/v) (Fig. 3Aa). The regression model of CGA (Y₂) revealed a positive relationship with ethanol concentration (X₂) (Fig. 3Ab). However, extremely high temperatures (X₁) compromised CGA extraction. CGA and its derivatives are transformed by isomerization and hydroxylation, or degraded to caffeic and quinic acid, by heat [37,38]. This explains why the yield of CGA decreased at the upper temperature. The regression model for the ginsenosides (Y₃₋₆) positively correlated with both temperature (X₁) and the ethanol ratio (X₂) (Fig. 3Ac-f). Lee et al. reported similar results; Rg₁ extractability from RG increased at a higher ethanol concentration and temperature [11].

The goodness-of-fit was tested by calculating the coefficient of multiple determinations (R^2), the adjusted coefficient of multiple determinations (adj. R^2), and the coefficient of variation (CV). All regression models were significant ($P < 0.0001$), thus with R^2 values of 0.800–0.993 and CVs of 3.94–9.62%. However, even if a best-fit model is optimized, regression analysis of individual responses may not yield the best results. For this reason, some models, such as Rg₁ (Y₄), differed in terms of the predicted and experimental values (Fig. 3Bd). In contrast, one model for Rb₁ (Y₅) exhibited low variance and data very similar to the experimental findings [39]. This regression model evidenced a strong correlation between the predicted and experimental values, and was thus more accurate than the other models (Fig. 3Be).

3.3. Optimization and validation of predicted values

Based on the regression models described above, the combinations of variables predicted to result in yields of at least 20% for dry matter, 0.35 mg/g dry extract for CGA, and 85 mg/g dry extract for the four ginsenosides are shown in Supplementary Fig. 1. A temperature of 80 °C and an ethanol concentration 60% were the conditions that allowed the production of the target yields. To validate the models, the predicted and observed values under these conditions (temperature 80 °C, ethanol concentration 60%) are shown in Supplementary Table 2. The following six responses were predicted: dry matter yield (Y₁), 21.11%; CGA yield (Y₂), 0.36 mg/g dry extract; Re yield (Y₃), 34.68 mg/g extract; Rg₁ yield (Y₄), 15.08 mg/g extract; Rb₁ yield (Y₅), 13.75 mg/g extract; and Rd yield (Y₆), 24.36 mg/g extract. The corresponding observed values were as follows: dry matter yield (Y₁), 25.67 ± 0.28%; CGA yield (Y₂), 0.24 ± 0.00 mg/g dry extract; Re yield (Y₃), 36.98 ± 1.60 mg/g

Table 2
Dry-matter yields and bioactive compound levels in pilot-scale extracts.

Extract	Dry-matter yield (% w/w)	Chlorogenic acid (mg/g)	Ginsenoside Re (mg/g)	Ginsenoside Rg ₁ (mg/g)	Ginsenoside Rb ₁ (mg/g)	Ginsenoside Rd (mg/g)
sHCG	35.5	0.383 ± 0.018 ^c	34.2 ± 0.4 ^c	9.7 ± 0.8 ^c	10.2 ± 0.1 ^b	24.2 ± 0.4 ^b
RG	38.2	0.019 ± 0.001 ^a	3.0 ± 0.0 ^a	6.4 ± 0.0 ^b	10.2 ± 0.4 ^b	0.7 ± 0.0 ^a
WG	54.4	0.079 ± 0.004 ^b	5.0 ± 0.4 ^b	4.8 ± 0.2 ^a	5.7 ± 0.4 ^a	0.5 ± 0.0 ^a

Different lowercase letters in the same column indicate significant differences between extracts ($P < 0.05$). sHCG, short-term hydroponic-cultured ginseng; RG, red ginseng; WG, white ginseng.

extract; Rg₁ yield (Y_4), 12.92 ± 0.48 mg/g extract; Rb₁ yield (Y_5), 11.95 ± 0.18 mg/g extract; and Rd yield (Y_6), 25.23 ± 0.54 mg/g extract. The observed and predicted values were compared to determine the reliability of the RSM. According to Dominguez et al., observed and predicted values are considered similar if the relative significant difference (RSD) is <10% [40]. The RSDs ranged from 0.28% to 4.33%, indicating that the predicted and observed values were similar.

3.4. Pilot-scale production of sHCG extracts

Pilot-scale extraction tests were carried out next. Analysis of samples collected during the first step of sHCG extraction yielded the following results: extraction yield, 25.4%; CGA, 0.27 mg/g extract; Re, 40.97 mg/g extract; Rg₁, 15.66 mg/g extract; Rb₁, 11.41 mg/g extract; and Rd, 34.08 mg/g extract. The pilot-scale production data were similar to the laboratory-scale results in Section 3.3.

Three sequential extractions were performed to maximize the yields. The contents of CGA and the four ginsenosides in the final pilot-scale product are shown in Table 2. The CGA content was 4.8- and 20.2-fold higher in the sHCG extract compared to the WG and RG extracts, respectively ($P < 0.05$). Chung et al. reported that the CGA content is 4.6- to 17.0-fold higher in leaves compared to roots [11]. Therefore, the abundant CGA in sHCG is attributable to its shoot parts, including stems and leaves. In addition, the sHCG extract had significantly higher Re, Rg₁, and Rd contents than did the WG and RG extracts. This result might be came from the existence of leaves, which have higher ginsenoside contents than roots [41,42]. Also, the Rb₁ concentration in the sHCG extract was approximately two-fold that in the WG extract. On the contrary, the Rb₁ content did not differ significantly between sHCG and RG extracts ($P > 0.05$). There are more than 100 ginsenoside derivatives,

which can be interconverted by heat and acidity [43,44]. Malonyl ginsenoside Rb₁, which is present in WG, is heat labile. Heating causes demalonylation of malonyl Rb₁ and its conversion to ginsenoside Rb₁ [45]. For this reason, the Rb₁ content in RG extracts may be similar to that in sHCG extracts.

3.5. Inhibitory effects of sHCG extracts on TNF- α /IFN- γ -induced production of TARC/CCL17 in HaCaT cells

The inhibitory effects of pilot-scale sHCG extracts containing large amounts of CGA and the four ginsenosides (Table 2) on AD were evaluated in terms of TNF- α /IFN- γ -induced production of the proinflammatory cytokine TARC/CCL17 in HaCaTs. According to the MTT assay results, the highest non-toxic concentration of the extracts was 50 μ g/mL (Fig. S2). Treatment of HaCaTs with TNF- α /IFN- γ increased the production of TARC/CCL17 to 368.05 ± 17.79 pg/mL, which was decreased by the sHCG extract (50 μ g/mL) to 144.12 ± 8.59 pg/mL (60.84% inhibition). Dexamethasone was used as a positive control because it has been used as a treatment for atopic dermatitis [46]. Dexamethasone also reduced TNF- α /IFN- γ -induced TARC/CCL17 production (214.18 ± 12.20 pg/mL, 41.81% inhibition), but the ability of the sHCG extract to reduce TARC/CCL17 production was significantly higher than that of 20 μ M dexamethasone (Fig. 4A). At 25 and 50 μ g/mL, the sHCG extract decreased TNF- α /IFN- γ -induced production of TARC/CCL17 in a dose-dependent manner. Again at 50 μ g/mL concentration, the ability of the sHCG extract was significantly higher than that of 20 μ M dexamethasone (Fig. 4B). Together, these results suggest that, compared to WG and RG, sHCG extracts containing significantly higher amounts of CGA, and the four ginsenosides, may have AD prevention effects. In this study, ginsenosides and phenolic compounds were principally focused on, because these are particularly abundant in shoots [11] and effective against AD [47].

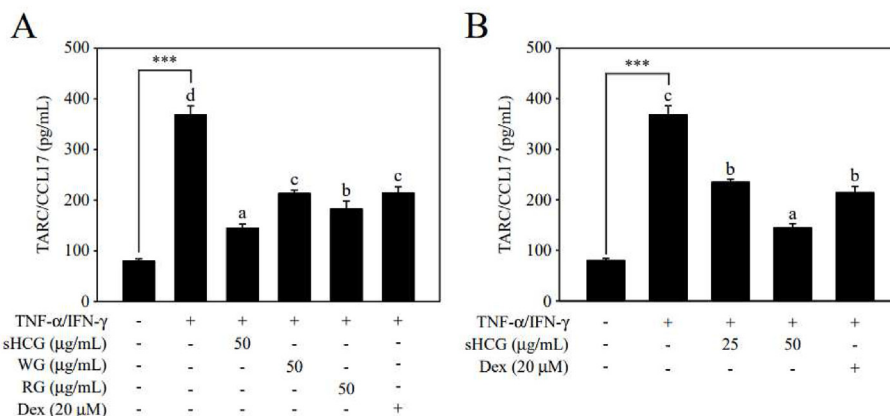


Fig. 4. Inhibitory effects of extracts from pilot-scale ginseng production on AD-associated chemokine TARC/CCL17 production in HaCaTs. (A) Inhibitory effects of 50 μ g/mL extracts from sHCG, WG, and RG on TNF- α /IFN- γ -induced TARC/CCL17 expression in HaCaTs. Dexamethasone (20 μ M) was used as a positive control. (B) Inhibitory effects of sHCG and dexamethasone on TNF- α /IFN- γ -induced TARC/CCL17 expression in HaCaTs, showing dose-dependency. Asterisks indicate significant differences from the control (** $P < 0.01$). Different lowercase letters indicate significant differences between TNF- α /IFN- γ treatment groups ($P < 0.05$).

However, in addition to ginsenosides and phenolic compounds, ginseng contains various other active ingredients, including polysaccharides [48] and gintonin [49]. Therefore, further studies are needed to determine whether such alternative active ingredients contribute to the beneficial effects of sHCG.

4. Conclusion

In this study, the optimal conditions for producing sHCG extracts rich in CGA, Re, Rg₁, Rb₁, and Rd were investigated. Pilot-scale extraction of sHCG, WG, and RG showed that the sHCG extracts had greater therapeutic potential for AD than WG and RG extracts. Our results suggest that sHCG extracts are potential candidates for ecologically sound production of anti-AD agents. Further studies are necessary to investigate whether these sHCG extracts prevent and/or reduce AD symptoms in animals or humans.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jgr.2021.10.006>.

References

- [1] Kim JS. Investigation of phenolic, flavonoid, and vitamin contents in different parts of Korean ginseng (*Panax ginseng* CA Meyer). *Prev Nutr Food Sci* 2016;21:263.
- [2] Chung SI, Kang MY, Lee SC. In vitro and in vivo antioxidant activity of aged ginseng (*Panax ginseng*). *Prev Nutr Food Sci* 2016;21:24–30.
- [3] Saba E, Jeong D, Irfan M, Lee YY, Park SJ, Park CK, Rhee MH. Anti-inflammatory activity of Rg3-enriched Korean red ginseng extract in murine model of sepsis. *Evid Based Complement Alternat Med* 2018;2018:6874692.
- [4] Lorz LR, Kim MY, Cho JY. Medicinal potential of *Panax ginseng* and its ginsenosides in atopic dermatitis treatment. *J Ginseng Res* 2020;44:8–13.
- [5] Kim H, Park CW, Cho SH. The beneficial effect of Korean red ginseng extract on atopic dermatitis patients: an 8 weeks open, noncomparative clinical study. *Ann Dermatol* 2018;30:304–8.
- [6] Lee KG, Son SW. Efficacy of Korean red ginseng in the treatment of atopic dermatitis. *J Ginseng Res* 2011;35:149–54.
- [7] Samukawa K, Izumi Y, Shiota M, Nakao T, Osada Oka M, Miura K, Iwao H. Red ginseng inhibits scratching behavior associated with atopic dermatitis in experimental animal models. *J Pharmacol Sci* 2012;118:391–400.
- [8] Kee JY, Jeon YD, Kim DS, Han YH, Park J, Youn DH, Kim SJ, Ahn KS, Um JY, Hong SH. Korean red ginseng improves atopic dermatitis-like skin lesions by suppressing expression of proinflammatory cytokines and chemokines in vivo and in vitro. *J Ginseng Res* 2017;41:134–43.
- [9] Sohn EH, Jang SA, Lee CH, Jang KH, Kang SC, Park HJ, Pyo S. Effects of Korean red ginseng extract for the treatment of atopic dermatitis-like skin lesions in mice. *J Ginseng Res* 2011;35:479–86.
- [10] Christensen LP. Ginsenosides: chemistry, biosynthesis, analysis, and potential health effects. *Adv Food Nutr Res* 2008;55:1–99.
- [11] Lee JW, Mo EJ, Choi JE, Jo YH, Jang H, Jeong JY, Jin Q, Chung HN, Hwang BY, Lee MK. Effect of Korean Red Ginseng extraction conditions on antioxidant activity, extraction yield, and ginsenoside Rg1 and phenolic content: optimization using response surface methodology. *J Ginseng Res* 2016;40:229–36.
- [12] Kim GS, Lee SE, Noh HJ, Kwon H, Lee SW, Kim SY, Kim YB. Effects of natural bioactive products on the growth and ginsenoside contents of *Panax ginseng* cultured in an aeroponic system. *J Ginseng Res* 2012;36:430–41.
- [13] Lee JY, Yang H, Lee TK, Lee CH, Seo JW, Kim JE, Kim SY, Park JHY, Lee KW. A short-term, hydroponic-culture of ginseng results in a significant increase in

- the anti-oxidative activity and bioactive components. *Food Sci Biotechnol* 2020;29:1007–12.
- [14] Hu JL, Nie SP, Huang DF, Li C, Xie MY. Extraction of saponin from *Camellia oleifera* cake and evaluation of its antioxidant activity. *Int J Food Sci Technol* 2012;47:1676–87.
- [15] Vuong QV, Hirun S, Chuen TL, Goldsmith CD, Murchie S, Bowyer MC, Phillips PA, Scarlett CJ. Antioxidant and anticancer capacity of saponin-enriched *Carica papaya* leaf extracts. *Int J Food Sci Technol* 2015;50:169–77.
- [16] Wei L, Zhang W, Yin L, Yan F, Xu Y, Chen F. Extraction optimization of total triterpenoids from *Jatropha curcas* leaves using response surface methodology and evaluations of their antimicrobial and antioxidant capacities. *Electron J Biotechnol* 2015;18:88–95.
- [17] Hijnen D, de Bruin Weller M, Oosting B, Lebre C, de Jong E, Bruijnzeel Koomen C, Knol E. Serum thymus and activation-regulated chemokine (TARC) and cutaneous T cell-attracting chemokine (CTACK) levels in allergic diseases: TARC and CTACK are disease-specific markers for atopic dermatitis. *J Allergy Clin Immunol* 2004;113:334–40.
- [18] Kim DK, Baik MY, Kim HK, Hahm YT, Kim BY. Standardization of ginseng processing for maximizing the phytonutrients of ginseng. *Food Sci Biotechnol* 2013;22:221–6.
- [19] Park SH, Lee CH, Lee JY, Yang H, Kim JH, Park JHY, Kim JE, Lee KW. Topical application of 7, 3', 4'-trihydroxyisoflavone alleviates atopic dermatitis-like symptoms in NC/Nga mice. *Planta Med* 2020;86:190–7.
- [20] Mondolot L, La Fisca P, Buatois B, Talansier E, De Kochko A, Campa C. Evolution in caffeoylquinic acid content and histolocalization during *Coffea canephora* leaf development. *Ann Bot* 2006;98:33–40.
- [21] Franklin G, Dias ACP. Chlorogenic acid participates in the regulation of shoot, root and root hair development in *Hypericum perforatum*. *Plant Physiol Biochem* 2011;49:835–42.
- [22] Tsang MS, Jiao D, Chan BC, Hon KL, Leung PC, Lau C, Wong EC, Cheng L, Chan CK, Lam CW. Anti-inflammatory activities of pentaherbs formula, berberine, gallic acid and chlorogenic acid in atopic dermatitis-like skin inflammation. *Molecules* 2016;21:519.
- [23] Cho WC, Chung WS, Lee SK, Leung AW, Cheng CH, Yue KK. Ginsenoside Re of *Panax ginseng* possesses significant antioxidant and antihyperlipidemic efficacies in streptozotocin-induced diabetic rats. *Eur J Pharmacol* 2006;550:173–9.
- [24] Park HJ, Byeon HE, Choi KW, Rhee DK, Lee KR, Pyo SN. Inhibitory effects of ginsenoside Rb1 on atopic dermatitis-like skin lesions in mice. *J Ginseng Res* 2010;34:363–8.
- [25] Yang Z, Chen A, Sun H, Ye Y, Fang W. Ginsenoside Rd elicits Th1 and Th2 immune responses to ovalbumin in mice. *Vaccine* 2007;25:161–9.
- [26] Zhang YX, Wang L, Xiao EL, Li SJ, Chen JJ, Gao B, Min GN, Wang ZP, Wu YJ. Ginsenoside-Rd exhibits anti-inflammatory activities through elevation of antioxidant enzyme activities and inhibition of JNK and ERK activation in vivo. *Int Immunopharm* 2013;17:1094–100.
- [27] Yamanaka K, Mizutani H. The role of cytokines/chemokines in the pathogenesis of atopic dermatitis. *Curr Probl Dermatol* 2011;41:80–92.
- [28] Nakajima S, Nomura T, Common J, Kabashima K. Insights into atopic dermatitis gained from genetically defined mouse models. *J Allergy Clin Immunol* 2019;143:13–25.
- [29] Kim BE, Leung DY. Significance of skin barrier dysfunction in atopic dermatitis. *Allergy Asthma Immunol Res* 2018;10:207.
- [30] Vestergaard C, Kirstejn N, Gesser B, Mortensen JT, Matsushima K, Larsen CG. IL-10 augments the IFN- γ and TNF- α induced TARC production in HaCaT cells: a possible mechanism in the inflammatory reaction of atopic dermatitis. *J Dermatol Sci* 2001;26:46–54.
- [31] Werfel T. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. *J Invest Dermatol* 2009;129:1878–91.
- [32] Vestergaard C, Bang K, Gesser B, Yoneyama H, Matsushima K, Larsen CG. A Th2 chemokine, TARC, produced by keratinocytes may recruit CLA+ CCR4+ lymphocytes into lesional atopic dermatitis skin. *J Invest Dermatol* 2000;115:640–6.
- [33] Wakugawa M, Nakamura K, Kakinuma T, Tamaki K. CC chemokine receptor 4 as a possible target for therapy of atopic dermatitis. *Drug News Perspect* 2002;15:175.
- [34] Zhao JL, Zhang M, Zhou HL. Microwave-Assisted extraction, purification, partial characterization, and bioactivity of polysaccharides from *Panax ginseng*. *Molecules* 2019;24.
- [35] Wang R, Chen P, Jia F, Tang J, Ma F. Optimization of polysaccharides from *Panax japonicus* C.A. Meyer by RSM and its anti-oxidant activity. *Int J Biol Macromol* 2012;50:331–6.
- [36] Dai CY, Liu PF, Liao PR, Qu Y, Wang CX, Yang Y, Cui XM. Optimization of flavonoids extraction process in *Panax notoginseng* stem leaf and a study of antioxidant activity and its effects on mouse melanoma B16 cells. *Molecules* 2018;23.
- [37] Li YJ, Zhang CF, Ding G, Huang WZ, Wang ZZ, Bi YA, Xiao W. Investigating the thermal stability of six caffeoylquinic acids employing rapid-resolution liquid chromatography with quadrupole time-of-flight tandem mass spectrometry. *Eur Food Res Tech* 2015;240:1225–34.
- [38] Zanoelo EF, Beninca C. Chemical kinetics of 5-o-caffeoylquinic acid in superheated steam: effect of isomerization on mate (*Ilex paraguariensis*) manufacturing. *J Agric Food Chem* 2009;57:11564–9.
- [39] Hadidi M, Ibarz A, Pagan J. Optimisation and kinetic study of the ultrasonic-assisted extraction of total saponins from alfalfa (*Medicago sativa*) and its

- bioaccessibility using the response surface methodology. *Food Chem* 2020;309:125786.
- [40] Dominguez R, Zhang L, Rocchetti G, Lucini L, Pateiro M, Munekata PES, Lorenzo JM. Elderberry (*Sambucus nigra* L.) as potential source of antioxidants. Characterization, optimization of extraction parameters and bioactive properties. *Food Chem* 2020;330:127266.
- [41] Kang OJ, Kim JS. Comparison of ginsenoside contents in different parts of Korean ginseng (*Panax ginseng* CA Meyer). *Prev Nutr Food Sci* 2016;21:389.
- [42] Shi W, Wang Y, Li J, Zhang H, Ding L. Investigation of ginsenosides in different parts and ages of *Panax ginseng*. *Food Chem* 2007;102:664–8.
- [43] Lee SM, Bae BS, Park HW, Ahn NG, Cho BG, Cho YL, Kwak YS. Characterization of Korean red ginseng (*Panax ginseng* meyer): history, preparation method, and chemical composition. *J Ginseng Res* 2015;39:384–91.
- [44] Shin BK, Kwon SW, Park JH. Chemical diversity of ginseng saponins from *Panax ginseng*. *J Ginseng Res* 2015;39:287–98.
- [45] Nam KY. The comparative understanding between red ginseng and white ginsengs, processed ginsengs (*Panax ginseng* CA Meyer). *J Ginseng Res* 2005;29:1–18.
- [46] Sung Y-Y, Yoon T, Jang S, Kim HK. *Forsythia suspensa* suppresses house dust mite extract-induced atopic dermatitis in NC/Nga mice. *PLoS One* 2016;11:e0167687.
- [47] Lee HS, Park JW, Kwon OK, Lim Y, Kim JH, Kim SY, Zamora N, Rosales K, Choi S, Oh SR. Anti-inflammatory effects of ethanol extract from the leaves and shoots of *Cedrela odorata* L. in cytokine-stimulated keratinocytes. *Exp Ther Med* 2019;18:833–40.
- [48] Lee SJ, In G, Han ST, Lee MH, Lee JW, Shin KS. Structural characteristics of a red ginseng acidic polysaccharide rhamnogalacturonan I with immunostimulating activity from red ginseng. *J Ginseng Res* 2020;44:570–9.
- [49] Cho HJ, Choi SH, Kim HJ, Lee BH, Rhim H, Kim HC, Hwang SH, Nah SY. Bioactive lipids in gintonin-enriched fraction from ginseng. *J Ginseng Res* 2019;43:209–17.