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

Tae Kyung Lee a,1, Ji Yun Lee a,1, Yeon-Jin Cho c, Jong-Eun Kim d, Seo Yeong Kim e, Jung Han Yoon Park a,b, Hee Yang c,e,**, Ki Won Lee a,b,c,e,*

a Department of Agricultural Biotechnology, Seoul National University, Seoul, Republic of Korea
b Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul, Republic of Korea
c Bio-MAX Institute, Seoul National University, Seoul, Republic of Korea
d Department of Food Science and Technology, Korea National University of Transportation, Jeungpyeong, Republic of Korea
e Advanced Institute of Convergence Technology, Seoul National University, Suwon, Republic of Korea

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.

© 2021 The Korean Society of Ginseng. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 μmol/m²/s. Silicate mineral water (pH 6.5–7.5) was sprayed twice daily. The 5-year-old WG (commonly cultivated 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 × 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 × 250 mm, 5 μm; Young Sin 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 (R² > 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-
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. 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 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 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].

2.6. 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 ± 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 shGC than in WG and RG. HPLC-UV quantification revealed a CGA content of 12.7 mg/100 g dry shGC, 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 shGC 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 shGC containing leaves and stems cultured in the early growth period than in conventionally grown ginseng and RG. In addition to CGA, 22 ginsenosides of shGC 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
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, Rg3, Rb1, and Rd can be found in shCG [13]. CGA and the four ginsenosides inhibited 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

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 (X1: 50 °C, 65 °C, and 80 °C) and ethanol concentration (X2: 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 (Y1), CGA (Y2), Re (Y3), Rg1 (Y4), Rb1 (Y5), and Rd (Y6) yields.

The dry matter yield (Y1) ranged from 16.9% to 27.4% (w/w). The lowest dry matter yield was obtained on run 11 (X1: 50 °C, X2: 70%), and the highest on run 25 (X1: 65 °C, X2: 0%). The CGA (Y2) yield ranged from 0.01 to 0.44 mg/g dry extract. The lowest CGA yield was observed on runs 12 (X1: 50 °C, X2: 0%), 17 (X1: 50 °C, X2: 0%), and 21 (X1: 50 °C, X2: 0%), and the highest on run 23 (X1: 65 °C, X2: 70%). For all extracts, the Re yield (Y3) was higher than those of the other ginsenosides (Y4, Y5, and Y6). This is consistent with our
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 ($R^2$).
Coded factors \(-1, 0, +1\) corresponding to independent variables are shown in parentheses.

The contents of the four ginsenosides were as follows: Re, 14.39 mg/g dry extract; Rb1, 2.32 \(\pm\) 0.00 mg/g dry extract; Rg1, 7.58 \(\pm\) 0.24 mg/g dry extract; and Rd, 0 \(\pm\) 0.00 mg/g dry extract. The yield of CGA decreased at the upper temperatures (X1) compromised CGA extraction. The highest levels of ginsenosides were obtained on run 5, at the highest temperature and ethanol concentration (X1: 80 °C, X2: 70%).

The goodness-of-fit was tested by calculating the coefficient of multiple determinations \(R^2\), the adjusted coefficient of multiple determinations \((\text{adj. } R^2)\), and the coefficient of variation \((\text{CV})\). 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 \((\text{Fig. 3Bd})\). 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 \((\text{Fig. 3Be})\).

### 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 CAg, 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 \(\left(\text{temperature 80 °C, ethanol concentration 60%}\right)\) were compared. The corresponding observed yield \(\left(Y_{\text{obs}}\right)\), predicted yield \(\left(Y_{\text{pred}}\right)\), and the relative percentage error \(\% \Delta E\) were used to calculate the coefficient of determination \((R^2)\) for the model predicting the yield of each ginsenoside.

\[
Y_{\text{obs}} = \sum_{i=1}^{n} Y_{\text{obs},i} \quad \text{and} \quad Y_{\text{pred}} = \sum_{i=1}^{n} Y_{\text{pred},i}
\]

\[
\% \Delta E = \frac{Y_{\text{obs}} - Y_{\text{pred}}}{Y_{\text{obs}}} \times 100\%
\]

\[
R^2 = \frac{\sum_{i=1}^{n} (Y_{\text{obs},i} - \bar{Y}_{\text{obs}})(Y_{\text{pred},i} - \bar{Y}_{\text{pred}})}{\sum_{i=1}^{n} (Y_{\text{obs},i} - \bar{Y}_{\text{obs}})^2}
\]

The coefficient of determination \((R^2)\) for the model predicting the yield of each ginsenoside was calculated for the predicted and observed yields under the conditions \(\left(\text{temperature 80 °C, ethanol concentration 60%}\right)\). The corresponding observed value \(\left(Y_{\text{obs}}\right)\), predicted yield \(\left(Y_{\text{pred}}\right)\), and the relative percentage error \(\% \Delta E\) were used to calculate the coefficient of determination \((R^2)\) for the model predicting the yield of each ginsenoside.
extract; Rg1 yield (Y4), 12.92 ± 0.48 mg/g extract; Rb1 yield (Y5), 11.95 ± 0.18 mg/g extract; and Rd yield (Y6), 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 shHG extracts

Pilot-scale extraction tests were carried out next. Analysis of samples collected during the first step of shHG 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 shHG extract compared to the WG and RG extracts, respectively (P < 0.05). Chung et al. reported that the CGA content did not differ significantly between extracts (P > 0.05). The WG and RG extracts had approximately two-fold higher CGA content than the shHG extract. On the contrary, the Rb1 content did not differ significantly between shHG and RG extracts (P > 0.05). Different lowercase letters in the same column indicate significant differences between extracts (P < 0.05). shHG, short-term hydroponic-cultured ginseng; RG, red ginseng; WG, white ginseng.

Table 2

| Extract | Dry-matter yield (% w/w) | Chlorogenic acid (mg/g) | Ginsenoside Re (mg/g) | Ginsenoside Rg1 (mg/g) | Ginsenoside Rb1 (mg/g) | Ginsenoside Rd (mg/g) |
|---------|--------------------------|-------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| shHG    | 35.5                     | 0.383 ± 0.018*          | 34.2 ± 0.4*           | 9.7 ± 0.8*            | 10.2 ± 0.1b           | 24.2 ± 0.4b           |
| RG      | 38.2                     | 0.019 ± 0.001*          | 3.0 ± 0.0*            | 6.4 ± 0.0b            | 10.2 ± 0.4b           | 0.7 ± 0.0a            |
| WG      | 54.4                     | 0.079 ± 0.004*          | 5.0 ± 0.4*            | 4.8 ± 0.2*            | 5.7 ± 0.4a            | 0.5 ± 0.0a            |

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

The inhibitory effects of pilot-scale shHG 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 extract was 50 μg/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 shHG extract (50 μg/mL) to 144.12 ± 8.59 pg/mL (60.84% inhibition). Dexmethasone was used as a positive control because it has been used as a treatment for AD. The inhibitory effects of pilot-scale shHG 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 TARC/CCL17 in HaCaTs. According to the MTT assay results, the highest non-toxic concentration of the extract was 50 μg/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 shHG extract (50 μg/mL) to 144.12 ± 8.59 pg/mL (60.84% inhibition). Dexmethasone was used as a positive control because it has been used as a treatment for AD.

**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 μg/mL extracts from shHG, WG, and RG on TNF-α/IFN-γ-induced TARC/CCL17 expression in HaCaTs. Dexmethasone (20 μM) was used as a positive control. (B) Inhibitory effects of shHG and dexmethasone 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 poly-
 saccharides [48] and gintonin [49]. Therefore, further studies are needed to determine whether such alternative active ingredients contribute to the beneficial effects of shGC.

4. Conclusion

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

Acknowledgments

This work was supported by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry, and Fisheries via the High Value-Added Food Technology Development Program (no. 116030-3) from the Ministry of Agriculture, Food and Rural Affairs, and by a National Research Foundation of Korea (NRF) grant (no. 2018R1A2A1A05078707) and K-BIO KIUPI Center program (no. 2020M3H1A1073034) from Ministry of Science and ICT, and by the BK21 Plus Program of the Department of Agricultural Biotechnology from Seoul National University.

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. Ginsenoside-Rd exhibits anti-inflammatory activities through elevation of antioxidant enzymes and inhibition of JNK and ERK activation in vivo. Int Immunopharmacol 2013;17:1094–100.
[9] Yamanake K, Mizutani H. The role of cytokines/chemokines in the patho-
gensis of atopic dermatitis. Curr Probl Dermatol 2011;41:80–92.
[10] Nakajima S, Nomura T, Common J, Kabashima K. Insights into atopic derma-
titis gained from genetically defined mouse models. J Allergy Clin Immunol 2019;143:13–25.
[11] Kim BE, Leung DY. Significance of skin barrier dysfunction in atopic derma-
titis. Allergy Asthma Immunol Res 2018;10:207.
[12] 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;26:26–31.
[13] Vestergaard C, Bang K, Genger B, Yoneyama H, Matsuhashi K, Larsen CG. Th2 cytokine, TARC, produced by keratinocytes may recruit CLA-CCR4 lymphocytes into lesional atopic dermatitis skin. J Invest Dermatol 2000;115:640–6.
[14] 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.
[15] Zhao J, Zhang M, Zhou HL. Microwave-Assisted extraction, purification, partial characterization, and bioactivity of polysaccharides from Panax ginseng. Molecules 2019;24:26.
[16] Wang R, Chen P, Jia F, Tang J, Ma F. Optimization of polysaccharides from Panax japonicus CA Meyer by RSM and its anti-oxidant activity. Int J Biol Macromol 2012;50:311–6.
[17] Dai CQ, Liu PF, Liao PR, Qi Y, Wang CX, Yang Y, Cui XM. Optimization of flavo-
vids extraction process in Panax notoginseng stem leaf and a study of antioxidant activity and its effects on mouse melanoma B16 cells. Molecules 2018;23.
[18] Li YJ, Zhang CF, 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.
[19] Zanoelo EF, Beninca C. Chemical kinetics of 5-o-caffeoylquinic acid in super-
heated steam: effect of isomerization on mate (Ilex paraguariensis) manufacturing. J Agric Food Chem 2009;57:11564–9.
[20] Hadidi M, Ibarz A, Fagan J. Optimization 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, Rochetti 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;330:67266.

[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 NC, Cho BC, 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.