A Study on Antioxidative and Antioxidative Effects of 
Codonopsis lanceolate and Platycodon grandiflourum Extracts

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Abstract

The effectiveness of 70% ethanol (Ethanol, EtOH) extract of Codonopsis lanceolate and Platycodon grandiflourum was investigated to verify its applicability as a functional cosmetic material. Antioxidant activity of antioxidant, total flavonoid content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and human dermal fibroblast (HDF) experiments were conducted to confirm the effect of collagen. Antioxidant activities of Codonopsis lanceolate and Platycodon grandiflourum extracts were increased by concentration of polyphenol and polabonoid effect, and no toxicity was observed in Human Dermal Fibroblast (HDF) cells. It was confirmed that collagen production was further increased in Duckweed extract. Dodeca and bellflower extracts are considered to be valuable as various functional cosmetic materials having antioxidative effect and anti-aging wrinkle improvement effect.

Keywords: Codonopsis lanceolate, Platycodon grandiflourum, Anti-oxidant, Cosmetic material, Collagen

Introduction

Codonopsis lanceolate is a wild herbaceous perennial herb that is widely used for general food in Korea [1], China and Japan. It is widely used for food and medicine due to its unique taste and aroma. Platycodon grandiflourum is an alimentary food used for food such as Codonopsis lanceolate[2]. Platycodin A, C, and D, which are triterpenoid saponins, are known to be the main ingredients. In addition, they contain inulin, betulin, stigmasterol, carbohydrate and fiber. Codonopsis lanceolate and Platycodon grandiflourum are the root of perennial herbaceous plant belonging to Campanulaceae and widely grown in China and Japan as Korea, and recent cultivation area has been expanded due to increased consumption of food and pharmacological health food [3,4]. Codonopsis lanceolate has sterol, triterpenoid, cycloartenol, N-formylharman, 1-carbomethoxy-β-carboline, perloyrine, norharman and volatile flavor components and has been reported to have pharmacological effects such as serum lipid reduction and antioxidant effects. Codonopsis lanceolate has long been used for food [5] because of its unique flavor and aroma. Platycodon grandiflourum contains triterpenoid saponins, carbohydrates and fibrin. It has been known to be effective for genomic, genetic, anti-ulcer, antipyretic, sedative, anti-inflammatory, hypotensive and tonsillitis [4]. The efficacy of Codonopsis lanceolate has been reported [6], serum lipid reduction [7], increased immunity [8], and antioxidant effects of cell wall materials [9,10]. Studies on Platycodon grandiflourum include mutagenic inhibition effects of general ingredients such as minerals, amino acids and fatty acids [11] and platycodon grandiflourum extracts [12], chemical composition and physiological activity [13], anti-carcinogenic and immunological activity of perennial Platycodon grandiflourum [14], anticholinergic action [15], hypoglycemic action [16] and improving cholesterol metabolism [17]. Many studies have already been carried out using Codonopsis lanceolate and Platycodon grandiflourum extract, and most of them have been reported as herbal medicine such as herbal medicine. Codonopsis lanceolate and Platycodon grandiflourum ethanol extracts for the production and development of functional cosmetics. Therefore, in this study, we will investigate the effect of 70% ethanol extract from Codonopsis lanceolate and Platycodon grandiflourum on the antioxidant effect. In this study, Codonopsis lanceolate and Platycodon grandiflourum will be developed as cosmetic functional materials.
Materials

Sample Preparation: In this study, *Platycodon grandiflorum* and *Codonopsis lanceolata* were purchased from pearls, dried, and then removed. The extracts were extracted with 70% ethanol extracted. Ethanol extraction was carried out by adding 70% ethanol at 10 times the volume of rosewood broth for 72 hours at room temperature. The extract was centrifuged at 8000 rpm for 20 minutes to separate the supernatant. Vacuum decompression was performed to remove ethanol as an extraction solvent. After concentrating with a decompressor and adding distilled water, it was stored at -70 °C for 24 hours, lyophilized, and then the powder was collected and stored.

Cell Line and Cell Culture: Human dermal fibroblast (HDF) cells were purchased from Korean Cell Line Bank, Korea and used in a high glucose Dulbecco’s modified Eagle’s medium (DMEM, Hyclone, USA) supplemented with 10% fetal bovine (Jeio Tech, Korea) supplemented with 1% penicillin/streptomycin (100 IU/50 μg/mL, Sigma-Aldrich) supplemented with serum (FBS; Sigma-Aldrich, USA) and kept at 37 °C and cultured.

Method

The Total polyphenol compound content measurement: The total polyphenol content was determined by modifying the Folin-Denis method of the Association of Official Agricultural Chemists (AOAC) to produce molybdenum blue when the Folin-Ciocalteau’s phenol reagent is reduced by phenolic compounds in the sample and quantified using the principle. Samples were diluted at concentrations of 5, 10, 15, and 20 μg/mL, and 400 μL of the sample and 400 μL of Folin-Ciocalteau’s phenol reagent were mixed and reacted at room temperature for 3 minutes. After reacting, 400 μL of 10% Na2CO3 was added and reacted in a dark room for 60 minutes. 200 μL of the supernatant was dispensed into a 96-well plate at 760 nm and absorbance was measured. Caffeic acid was used as a standard substance. The total polyphenol content in the sample was determined by substituting the absorbance value (Y axis) of the standard curve sample of Caffeic acid (0 to 100 μg/mL) for the concentration (X axis). The calibration curves were prepared for the standard concentration of the substance on the X axis and the peak area on the Y axis. The calibration curve for each polyphenol component was used as a standard curve showing linearity of the correlation coefficient ($R^2$) (Fig. 1).

The Total Flavonoid Content Measurement: Total flavonoid content was determined by Moreno et al. (2000) Method was modified and modified to measure. After diluting the sample to 5, 10, 15 and 20 μg/mL, 100 μL of the sample, 20 μL of 10% Al(NO3)3, 20 μL of 1M CH3COOK and 860 μL of ethanol were mixed in this order and left at room temperature for 40 minutes. The supernatant was submerged in a centrifuge (Fine PCR, Korea), and 200 μL of the supernatant was dispensed into a 96-well plate. Absorbance was measured at 415 nm. The average value was measured by repeated experiment three times under the same conditions, and the standard substance quercetin was used. The total flavonoid content in the sample was determined by substituting the absorbance value (Y axis) of the sample into the standard curve of quercetin (0 to 100 μg/mL) to determine the concentration (X axis). The calibration curves were prepared for each flavonoid component using the standard concentration and the peak area as the X axis and the Y axis, respectively. The calibration curve ($R^2$) showing the straight line showed good linearity of 0.9621 and used as a standard curve (Fig. 2).
DPPH Radical Scavenging Activity: DPPH radical scavenging activity was measured using the Blois (1958) method. After dilution of the samples by concentration, 180 μL of 10 mM DPPH solution and 20 μL of sample solution were mixed in a 96-well plate and reacted at 37 °C for 30 min in the shade state. Then, Synergy HT (BioTek Instruments, USA) The absorbance was measured. The test was repeated three times under the same conditions and the mean value was measured. As a positive control, ascorbic acid was used.

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\text{DPPH radical Scavenging} = \left( \frac{\text{O.D. at 517 nm of the group with extract}}{\text{O.D. at 517 nm of the group without extract}} \right) \times 100
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Cell Viability Measurement: To investigate the effect of the extract on cell viability, a neutral red (NR) assay was used. Human dermal fibroblast (HDF) cells were seeded at a density of 3 × 10^4 cells/well in a 96-well plate and cultured for 24 hours. The cells were diluted to 6.25, 12.5, 25, and 50 μg/And cultured for 48 hours. After 48 hours, the medium was replaced with culture medium supplemented with 1% NR solution (Sigma-Aldrich) in serum-free medium, incubated for 3 hours, and then incubated with 10% formaldehyde (Sigma-Aldrich, USA) was added to each well, and 100 μL of each solution was dispensed into each well and fixed for 20 minutes. NR desorbed solution (1% glacial acetic acid (Sigma-Aldrich, USA), 49% ethanol (Duksan, Korea) and 50% distilled water) HT, BioTek Instruments, USA) was used to measure the absorbance at 540 nm. The cell viability of this experiment was calculated according to the following equation.

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\text{Cell viability} = \left( \frac{\text{O.D. at 540 nm of the group with extract}}{\text{O.D. at 540 nm of the group without extract}} \right) \times 100
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Measurement of Collagen Production Promotion: HDF cells were cultured in 96-well plates at a concentration of 1 × 10^4 cells/well and allowed to stand on the bottom for 24 hours. After confirming cell adhesion, diluted samples were added and cultured for 48 hours. After incubation, the culture supernatant was transferred to a 50-mL 96-well plate, and 100 mL of carbonate coating buffer (Na2CO3 + NaHCO3 + 10% NaN3, pH 9.5) was added and fixed at 4 °C for 24 hours. After washing three times with 200 mL of PBS-T, 100 mL of blocking solution (PBS, 0.1% BSA) was blocked at 37 °C for 1 hour. After blocking, 100 mL of primary antibody (anti-collagen type I-Ab mouse IgG) diluted 1000-fold with blocking solution was added to each well and incubated at 37 °C for 1 hour. After washing three times with 200 mL of PBS-T, 100 mL of each anti-mouse IgG-antibody-conjugated secondary antibody (anti-mouse IgG-antibody) diluted in 4000-. After 1 hour, the plate was washed three times with 200 mL of PBS-T, and the substrate was treated with pH 9.8, p-nitrophenyl phosphate in 9.7% diethanolamine buffer, and 0.5 mM MgCl2 in 200 mL of each well. And the absorbance was measured at a wavelength of 405 nm.

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\text{Collagen production promotion} = \left( \frac{\text{O.D. at 540 nm of the group with extract}}{\text{O.D. at 540 nm of the group without extract}} \right) \times 100
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Statistical Processing: Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) window version 17.0 (IBM, USA). The results were expressed as mean ± standard deviation (M ± SD) Statistical significance was tested by Student’s t-test and p<0.05 was considered statistically significant. All experiments were carried out three times or more independently under the same conditions, and the experimental results were obtained and analyzed.

Result and Discussion

1. Total Polyphenol Content Results: Natural antioxidants isolated from various natural products include ascorbic acid, tocopherols, carotenoids, maillard reaction products, amino acids, peptides, phospholipids, and polyphenols and flavonoids. To determine the total polyphenol content of the 70% ethanol extracts, the concentrations of 5, 10, 15 and 20 μg/mL were measured. The total polyphenol content of each extract was calculated from the calibration curves prepared using caffeic acid as a standard solution. As a result, the total polyphenol content of CIE extract at 20 μg/mL was 150.3 mg/100 g and that of PGE extract was 74.9 mg/100 g (Fig. 3). The total polyphenol contents in the bellflower extracts were increased in a concentration dependent manner. The results were higher than those of the 70% ethanol extracts. The extraction with organic solvents was more effective than the extraction with distilled water as the solvent. The solubility of the polar solvent is considered to be high.
Figure 3: Total polyphenol content of *Codonopsis lanceolata* et *Platycodon grandiflorum* extract. Values represent mean ± standard deviation of three measurements. CIE: *Codonopsis lanceolata* extract, PGE: *Platycodon grandiflorum* extract

2. Total Flavonoids Content Results

Figure 4: Total flavonoid content of *Codonopsis lanceolata* et *Platycodon grandiflorum* extract. Values represent mean ± standard deviation of three measurements. CIE: *Codonopsis lanceolata* extract, PGE: *Platycodon grandiflorum* extract

3. DPPH radical Scavenging activity Results:
Free radicals are known to be the cause of aging reaction with human body proteins and lipids. Free radical scavenging ability by DPPH method is widely used for verifying the antioxidant ability of antioxidant by reactive oxygen species (ROS). Figure 5 shows the results of DPPH radical scavenging activity. The ascorbic acid used as a positive control showed a radical scavenging activity of 120% at a concentration of 1% and a CIE extract 0.5%, 109.0% of the radical scavenging activity, and 92.0% of the scavenging activity at the concentration of 0.5% of the PGE extract. Thus, it was confirmed that the radical scavenging activity of the CIE extract was higher than that of the PGE extract. As a result of measuring the DPPH radical scavenging activity, the DPPH scavenging activity showed a radical scavenging activity in a concentration dependent manner as the concentration of the extract increased.

Figure 5: The DPPH radical scavenging activity of of *Codonopsis lanceolata* et *Platycodon grandiflorum* extract. After treating *Codonopsis lanceolata* et *Platycodon grandiflorum* extract at 1, 10, 30 and 50 mg/mL, high density dependent DPPH radical scavenging activity was identified. The results are presented as the Mean ± S.D. of three independent experiments. CIE: *Codonopsis lanceolata* extract, PGE: *Platycodon grandiflorum* extract

4. Measurement of cell viability on HDF cells using Neutral red (NR) assay:
In order to investigate the effect of CIE and PGE extracts on the cell viability of human fibroblasts, HDF cells, the concentrations of CIE extract and PGE extracts at 5, 10, 20, 50, and 100 μg/mL with 70% ethanol and cultured for 48 hours to perform NR assay. In the case of CIE extract, the cell proliferation rate was increased with increasing concentrations of 5, 10, 20, 50, and 100 μg/mL. Cell survival rate was higher than 100%. The cell viability was found to be 119% for CIE and 101% for PGE extract at 100 μg/mL concentration of marigold and calendula extract (Fig. 6).

Figure 6: Effect of CIE et PGE extract on cell viability in HDF cells. Results is presented as mean ± SD, and three independent experiments were performed. The results are presented as the Mean ± S.D. of three independent experiments. CIE: *Codonopsis lanceolata* extract, PGE: *Platycodon grandiflorum* extract
5. Measurement of collagen production promotion: In order to observe the change of collagen production promoting ability of CIE and PGE extracts on HDF cells of the sample, CIE and PGE extracts were treated with HDF cells for 48 hours, and the culture supernatants were analyzed by ELISA (Enzyme-Linked Immunosorbent Assay). The results are shown in Figure 7.

Compared with the untreated control group, collagen production was promoted by CIE and PGE extracts. At the concentration of 50 μg/mL of CIE extract, the highest increase of collagen production was observed at 109.62%, and collagen was increased in a dose dependent manner (**p<.01, ***p<.001). Compared with the positive control group, vitamin C, the concentration of vitamin C at 20 μg/mL and the concentration of CIE and PGE extract of 20 μg/mL showed similar amounts of collagen production. These results suggest that CIE and PGE extracts may be useful as cosmetic materials containing CIE and PGE extract.

Figure 7: Effects of CIE and PGE extracts on the collagen secretion by HDF cells. Results are presented as mean ± SD, and three independent experiments were performed. The results are presented as the Mean ± S.D. of three independent experiments. CIE: Codonopsis lanceolata extract, PGE: Platycodon grandiflorum extract (**p<.01, ***p<.001)

Conclusion

In this study, the purpose of this study was to investigate the applicability of CIE and PGE extracts as cosmetic materials. The results of the antioxidative and skin cell activities of CIE and PGE extracts are as follows.

1. The 70% ethanol extract of CIE and PGE showed excellent antioxidative power against polyphenol, flavonoid and DPPH radical scavenging ability.

2. The cell viability and cytotoxicity of HDF cells were examined. The cytotoxicity was not observed in all the 70% ethanol extraction methods at all concentrations up to 5 ~ 100 μg/mL, and high survival rate was confirmed.

3. Collagen production was promoted in both extracts of CIE and PGE extracts. At the concentration of 100 μg/mL, the highest increase of collagen production was observed at 109.62%, and collagen increased in a dose dependent manner. Compared with the positive control group, vitamin C, a similar amount of collagen production was observed at a concentration of 20 μg/mL of vitamin C and 50 μg/mL of CIE extract. These results suggest that CIE and PGE extracts may be applicable as a cosmetic material containing CIE and PGE extract. Considering the results of the present study, CIE and PGE extracts showed antioxidant activity, cell viability and collagen effect. As a result, CIE and PGE extracts may be useful as functional cosmetic materials.

Ethical Clearance: Not required

Source of Funding: Nil

Conflict of Interest: Nil

REFERENCES


