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Supercritical fluid extract of *Angelica gigas* Nakai promotes collagen synthesis in human dermal fibroblast

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ABSTRACT

The extract of *Angelica gigas* Nakai by supercritical CO_2 promoted the expression of collagen synthesis-related proteins in human dermal fibroblast. The condition applied for the supercritical fluid extraction was 400 bar and 60°C. By the proteome expression profiling, both of type 1(α-2) collagen chain precursor and prolyl 4-hydroxylase were expressed 1.7 times more. The procollagen C-endopeptidase was also increased 1.5 times. Proteome analysis proved that *Angelica gigas* Nakai promoted proteome synthesis related to skin anti-aging in human dermal fibroblasts.

KEYWORDS

Angelica gigas Nakai;
Supercritical CO_2;
Skin anti-aging;
Collagen synthesis;
Human dermal fibroblast.

INTRODUCTION

Angelica gigas Nakai, which in Korea is referred to as 'Chamdanggui', is widely utilized as an important folk medicine, not only for the treatment of anemia but also as a sedative and anodyne agent. It has been reported to contain a variety of compounds, including coumarins, essential oils, and volatile flavors[1]. Decursin and decursin angelate, members of the coumarin family, can perform an important role in hydrogen peroxide scavenging[2], anti-tumor[3], and anti-platelet activities[4]. However, few studies have been conducted on the skin whitening and skin anti-aging properties of decursin and decursinol angelate.

Supercritical carbon dioxide is now well established as a solvent that can be utilized in the extraction of raw materials for cosmetic applications. This method has a number of advantages. The method generally allows for quicker penetration of solid samples than is the case with liquid solvents, due to higher diffusion rates, and this method also allows for the rapid transport of dissolved solutes from the sample matrix due to low viscosity. Almost no solvent residues are present in the products. It is also, of course, conducted at low extraction temperatures, which lessens the denaturalization of the bioactive natural products[5-8].

In the dermis, the regulation of fibroblast proliferation, differentiation, migration, and apoptosis is dependent on the integrity of the extra-cellular matrix (ECM), and age-associated matrix changes are probably determinants of alterations in dermal functions. The dermis layer is composed of connective tissue and blood

vessels. Dermal connective tissue contains collagen and elastin. Collagen fibers account largely for the volume of the skin and the bulk of its tensile strength, whereas elastic fibers are associated with skin's elasticity and resilience[9]. Collagen alterations are the most relevant changes occurring within the dermis in intrinsic and photo-aged skin. During the skin-aging process, collagen synthesis and enzymes involved in the post-translational processing of collagen are reduced[10]. Thus, reduced collagen contents have been suggested as a cause of the skin wrinkling observed in aged skin[11].

In this study, we made the extraction from *Angelica gigas* Nakai with supercritical CO_2. The anti-aging effect of the extract was observed on human dermal fibroblast cultures with proteome analysis. Proteome analysis based on 2-D PAGE was applied for the profiling of proteins related to collagen synthesis.

EXPERIMENTAL

Supercritical fluid extraction from *Angelica gigas* Nakai

10g of dried *Angelica gigas* Nakai, which was grown in Korea, were ground for extraction. For supercritical carbon dioxide extraction, an apparatus including a one-liter extractor was used. Ground *Angelica gigas* Nakai powder was filled and packed into the extractor. Liquid carbon dioxide was compressed and supplied to the extractor using a pump. The temperature of the compressed supercritical fluid was adjusted with the heat exchanger prior to its introduction to the extractor. Various combinations of supercritical carbon dioxide pressure and temperature were applied in an effort to optimize the extraction. After extraction, the pressure of the carbon dioxide-containing extract was reduced using the backpressure regulator. It was then separated into gaseous carbon dioxide and extracts in the separator. The liquid phase extract was harvested from the separator. The gaseous carbon dioxide was liquefied with the chiller and recycled via supply to the compression pump.

Cell cultures

Human dermal fibroblasts were purchased from the American Type Culture Collection (ATCC, CRL-1635). Fibroblasts were cultured in DMEM (WelGene lnc.) with 10% fatal bovine serum (Gibco), and 1% Antibiotic-antimycotic (Gibco). Cultures were prepared in a humidified CO_2-controlled (5%) incubator at 37°C. The cells were sub-cultured every seven days.

Cell viability assay (MTT assay)

An MTT assay was utilized to determine the ability of viable cells that convert a soluble tetrazolium salt, 3-(4,5-dimethyl-2-tetrazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), into an insoluble formazan precipitate. In brief, the fibroblasts were seeded into 96-well plates at 3×10^3 cells/well and allowed 24 hours for surface adhesion. The culture medium was removed after 24 hours, then exchanged with a new medium containing *Angelica gigas* Nakai extracts at variable concentrations from 0~100 mg/L. The cultures were then maintained for three days at 37°C in a CO_2 incubator. After 3 days of cultivation, the medium was removed. The cells were washed twice in PBS and incubated for 5 hours in MTT solution at 37°C. After incubation, the MTT solution was removed. The formazan crystals formed were dissolved with DMSO for 15 min. Cell viability was determined by measuring the optical density of the formazan solution at 540 nm[12].

Proteome analysis

Cells washed in PBS were isolated via centrifugation. Proteins were extracted by cell lysis. The lysis buffer solution was prepared with 7 M urea, 2 M thiourea, 4% CHAPS, 1% DTT, 2% Carrier ampholyte, 4% PIC, and 0.002% BPB. Cells in lysis buffer were sonicated for 1 min and maintained for 30 min at room temperature. The solution was centrifuged for 5 min at 4000×g. The supernatant was collected and preserved at -20°C. The amount of total protein extracted was measured via the modified Bradford method[13]. Bovine γ-globulin was utilized as the standard protein.

Immobiline Dry strips (13cm, pI 3-10L, GE Healthcare, and USA) were used with an IPGphore fixed-length strip holder. The strip was rehydrated for 12 h with rehydration solution and the sample proteome was injected simultaneously. The rehydration solution was prepared with 7 M urea, 2 M Thiourea, 2% CHAPS, 1% DTT, 2% Carrier ampholyte, 10% glycerol, 0.002% BPB. Isoelectric focusing was conducted

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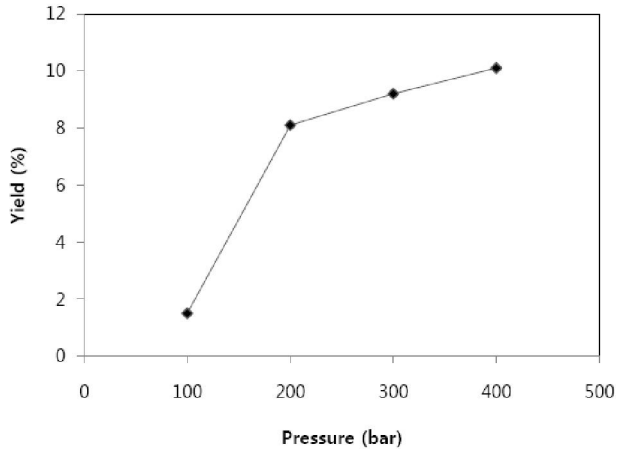

Figure 1 : Supercritical CO_2 extraction yield of *Angelica gigas* Nakai at various extraction pressures. The extraction temperature was maintained constant as 60°C

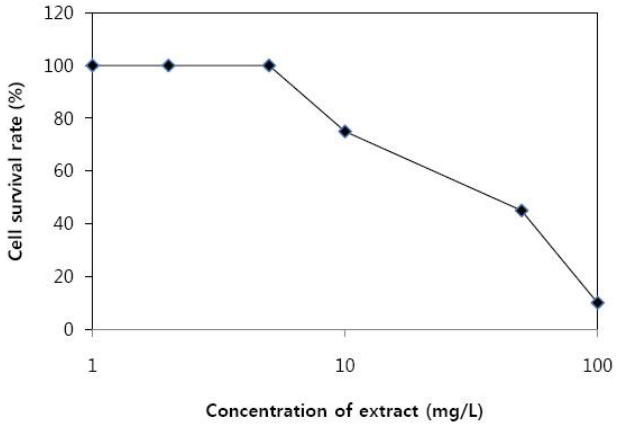

Figure 2 : Effects of *Angelica gigas* Nakai extracts on the cell viability of human dermal fibroblasts. Cell viabilities were observed after three days from dosing with various concentrations of *Angelica Gigas Nakai* extract

in IPGphore (Amersham Bioscience, Sweden). After 8 hours of rehydration, stepwise focusing was performed for 60 min at 500 V, 60 min at 2000 V, and then increased to 8000 V and maintained until no current change was observed[14]. Focused IPG strips were equilibrated for 15 min in a solution (7 M urea, 2 M Thiourea, 2% SDS, 50 mM Tris-HCl, 30% glycerol and 1% DTT), and then for an additional 15 min in the same solution containing 2.5% iodoacetamide rather than DTT[15]. After equilibration, the second dimension was run on 11.5% polyacrylamide homogenous gels (T-13%, C-2.5%, 18×24 cm)[16]. The gels were stained with silver nitrate[17]. The stained gel was scanned and the protein spot images were analyzed using 2D Elite (Amersham Biosciences, Sweden) image analysis software. The MALDI-tof MS analysis was made for some protein spots to make the proof identification.

RESULTS AND DISCUSSION

Extraction of *Angelica gigas* Nakai with supercritical CO_2

The efficiency of supercritical carbon dioxide extraction depends on many factors associated with the density of the supercritical fluid. Among them, pressure and temperature exert the most profound influence. The extraction efficiency of *Angelica gigas* Nakai was assessed by altering the pressures and temperatures of supercritical carbon dioxide. In order to determine the effects of extraction pressure, various supercritical carbon dioxide pressures, 100, 200, 300, and 400 bars, were applied at a constant temperature of 60°C. 400 bars was the highest pressure applicable using our system. At each different pressure, the extraction yield was evaluated. The extraction yield was calculated as the percentage of the supercritical fluid extract to the total mass of raw material. As is shown in figure 1, we determined that the extraction efficiency increased with increasing pressure. This is normally observed when the supercritical carbon dioxide is applied to the extraction of lipophilic compounds from plants. As the pressure increases, the density of the supercritical carbon dioxide also increases, which results in higher solvent power, allowing for more of extract to be dissolved out of the *Angelica gigas* Nakai powder[18]. Various supercritical carbon dioxide temperatures were tested at a constant pressure of 400 bars. The extraction efficiency increased with increasing temperature. The yield increase as the result of temperature differences, however, was not as profound as the yield increase attributed to differences in pressure. Due to the effects of pressure and temperature, the conditions for the extraction of *Angelica gigas* Nakai via supercritical carbon dioxide were at 400 bars, 60°C throughout this study.

Cytotoxic effects of *Angelica gigas* Nakai extracts on human dermal fibroblasts

The cytotoxic effects of *Angelica gigas* Nakai extracts differed with different extraction methods; hot water, 95% ethanol, and supercritical CO_2 fluid (300bar, 60°C) extractions were investigated against human skin

TABLE 1 : Spot volume of expressed proteins related to collagen synthesis on *Angelica gigas* Nakai extracts (5mg/L)-stimulated human dermal fibroblast

Group	Protein name	control	SCF
Collagen synthesis related proteins	Type 1(α-2) collagen chain precursor	7,011,723	12,471,633
	procollagen C-endopeptidase	17,738,024	25,937,052
	prolyl 4-hydroxylase	1,270,742	2,154,150

fibroblasts. An MTT assay was conducted with *Angelica gigas* Nakai extracts at various concentrations, in a dose range of 0.001~100 mg/L. According to the results of the MTT assay as shown in figure 2, the supercritical fluid extract evidenced no cytotoxic activities on fibroblasts at concentrations up to 5 mg/L. From this experimental result, the dose concentration of supercritical fluid extract in cultures of human dermal fibroblast was decided as 5 mg/L.

Promotion of collagen synthesis in human dermal fibroblast

Human skin fibroblasts dosed with 5 mg/L of *Angelica gigas* Nakai extracts were harvested after 48 hours. 2D-PAGE was made with protein samples extracted from the harvested cells. Protein profiling was conducted to compare the proteins expressed with and without extract. In the control gel, which was not treated with *Angelica gigas* Nakai extract, 485 spots were counted in the. In the gel used with the supercritical fluid extraction, 444 spots were identified. When compared with the control gel, 253 spots were found to match. 137 of these spots were up-regulated and 77 of the spots were down-regulated (Figure 3). Among the regulated proteins, proteins related to the collagen synthesis were identified via 2D-image analysis and MALDI-TOF. The collagen synthesis-related proteins were identified as follows: type 1(α-2) collagen chain precursor (pI 9.08, MW 129.7), procollagen C-endopeptidase (pI 7.40, MW 47.97), prolyl 4-hydroxylase (pI 5.49, MW 60.90).

TABLE 1 clearly shows that the dose of supercritical fluid extract increased the expression of 3 proteins related to the collagen synthesis in human dermal fibroblast. The type 1 collagen chain precursor and the propyl 4-hydroxylase were increased 1.7 times respectively. 1.5 times increase of procollagen C-endopeptidase was observed too. The propyl 4-hydroxylase is one of enzymes related to tropocollagen synthesis from protocollagen. The procollagen C-endopeptidase is the enzyme catalyzes collagen synthesis from procollagen by the limited proteolysis for the solubility reduction. Increases of these enzymes and collagen precursor are clear evidences for the promotion of collagen synthesis, one of important mechanisms for anti-aging, by the supercritical fluid extract of *Angelica gigas* Nakai. Besides the role of collagen synthesis-related proteins, many other mechanisms are involved for skin anti-aging. The interaction between extra-cellular matrix such as collagen and cell substance is as important as collagen synthesis. The interaction of fibroblasts is mediated by specific receptors on their surfaces[18-20].

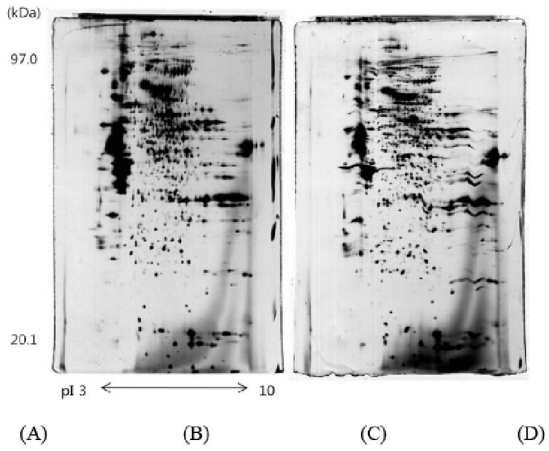

Figure 3 : 2D gel electrophoresis of total proteins extracted from human dermal fibroblast. Cells treated with and without 5 mg/L *Angelica gigas* Nakai extracts at 48 h; (left) control, (right) supercritical fluid extract

From the results of this study, *Angelica gigas* Nakai extracted with supercritical CO_2 fluid was found to promote collagen synthesis in human dermal fibroblasts, important mechanism to inhibit skin-aging. This was proved by the proteome analysis that *Angelica gigas* Nakai promoted proteome synthesis related to skin collagen synthesis in human dermal fibroblasts. Further studies to identify components involved in this promotion would be anticipated to develop the possible anti-aging agent.

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REFERENCES

[1] M.R.Kim, A.El-Aty, I.S.Kim, J.H.Shim; Journal of Chromatography A, **1116**, 259 **(2006)**.

[2] S.H.Lee, Y.S.Lee, S.H.Jung, K.H.Shin, B.K.Kim, S.S.Kang; Natural Product Sciences, **9**, 170 **(2003)**.

[3] S.H.Lee, Y.S.Lee, S.H.Jung, K.H.Shin, B.K.Kim, S.S.Kang; Archives of Pharmacal Research a Publication of the Pharmaceutical Society of Korea, **26**, 727 **(2003)**.

[4] Y.Y.Lee, S.H.Lee, J.L.Jin, H.S.Yun-Choi; Archives of Pharmacal Research a Publication of the Pharmaceutical Society of Korea, **26**, 723 **(2003)**.

[5] E.Vagi, B.Simandi, H.G.Daood, A.Deak, J.Sawinsky; Journal of Agricultural and Food Chemistry, **50**, 229 **(2002)**.

[6] H.Sovova, M.Sajfrtova, M.Bartlova, L.Opletal; The Journal of Supercritical Fluids, **30**, 213 **(2004)**.

[7] M.Arlorio, J.D.Coisson, F.Travaglia, F.Varsaldi, G.Miglio, G.Lombardi, A.Martelli; Food Research International, **38**, 1009 **(2005)**.

[8] J.C.de la Fuente, B.Oyarzun, N.Quezada, J.M.del Valle; Fluid Phase Equilibria, **247**, 90 **(2006)**.

[9] M.P.Brincat, Y.M.Baron, R.Galea; Climateric, **8**, 110 **(2005)**.

[10] A.Oikarinen; Photodermatol Photoimmunol Photomed, **7**, 3 **(1990)**.

[11] J.Varani, R.L.Warner, M.Gharaee-Kermani, S.H.Phan, S.Kang, J.Chung, Z.Wang, S.C.Datta, G.J.Fisher, J.J.Voorhees; The Journal of Investigative Dermatology, **114**, 480 **(2000)**.

[12] A.M.Sieuwert, J.G.M.Klijn, H.A.Peters, J.A.Foekens; European Journal of Clinical Chemistry and Clinical Biochemistry, **33**, 813 **(1995)**.

[13] M.M.Bredford; Analytical Biochemistry, **72**, 248 **(1976)**.

[14] A.Gorg, C.Obermaier, G.Boguth, A.Harder, B.Scheibe, R.Wildgruber, W.Weiss; Electrophoresis, **21**, 1037 **(2000)**.

[15] M.M.Sanders, E.T.Broconing; Analytical Biochemistry, **103**, 157 **(1980)**.

[16] B.Herbert, M.P.Molloy, K.L.Williams; Electrophoresis, **19**, 845 **(1998)**.

[17] B.S.Yoo, M.A.Yoo, Y.K.Song, S.Y.Byun; Biotechnology and Bioprocess Engineering, **12**, 662 **(2007)**.

[18] G.Segal, W.Lee, P.D.Arora, M.McKee, G.Downey, C.A.G.McCulloch; Journal of Cell Science, **114**, 119 **(2000)**.

[19] L.Moreau, S.Bordes, M.Jouandeaud, B.Closs; Cosmetics & Toiletries, **118**, 75 **(2003)**.

[20] W.Lee, C.A.G.McCulloch; Experimental Cell Research, **237**, 383 **(1997)**.