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Pectolinarigenin, an aglycone of pectolinarin, has more potent inhibitory activities on melanogenesis than pectolinarin

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ABSTRACT

Pectolinarin and pectolinarigenin have been reported to be major compounds in *Cirsium setidens*. In the present study, we demonstrated inhibitory effects of pectolinarin and pectolinarigenin from *C. setidens* on melanogenesis. Melanin synthesis was decreased in both pectolinarin- and pectolinarigenin-treated melan-a cells and in a reconstructed human skin model. However, pectolinarigenin treatment showed more potent inhibitory activity of melanin synthesis than did pectolinarin treatment. The concentrations of pectolinarin and pectolinarigenin in *C. setidens* water extracts were determined by HPLC. Unfortunately, the amount of pectolinarigenin of *C. setidens* water extract was lower than that of pectolinarin. To increase the pectolinarigenin content in *C. setidens* water extract, several component conversion methods were studied. Consequently, we identified that microwave irradiation under 1% acetic acid was an optimum sugar elimination method.

1. Introduction

Melanin is a natural pigment that is produced by a specialized group of cells known as melanocytes and melanoma cells and by the oxidation of the amino acid tyrosine followed by polymerization. It plays a key role in protecting cells from cytotoxic light and determines the skin color of mammals [1–3]. In the skin, melanin is synthesized to defend against UV damage through a complex process that involves various enzymes and a series of signal pathways [4]. The biosynthesis of melanin, called melanogenesis, begins with the oxidation of tyrosinase to DQ (DOPAquinone) via the intermediate DOPA (3,4-dihydroxyphenylalanine) [5]. Second, TRP-2 (tyrosinase-related protein 2) converts DQ into DOPAchrome and subsequently converts DOPAchrome into DHICA (indole 5,6-quinone 2-carboxylic acid) or DHI (5,6-dihydroxyindole). Finally, TRP-1 (tyrosinase-related protein 1) converts DHICA and forms eumelanins [6]. However, the overproduction of melanin causes medical problems such as melanoderma, post-inflammatory diseases and melasma [7,8]. Since the development of anti-melanogenic agents is an important goal in the clinical and cosmetic fields, many studies have focused on understanding the exact mechanism of melanogenesis. Traditionally, researchers tried to develop anti-melanogenic agents and have focused on small molecules or natural products that serve as inhibitors of tyrosinase, the rate-limiting enzyme in melanogenesis [9].

Cirsium setidens Nakai is a perennial plant of the aster family in the genus *Cirsium*. *C. setidens*, also known as “gondre” in English, is found mainly in the Kangwon province of Korea [10]. The young leaves and stems of *C. setidens* are rich in protein, calcium, and vitamin A and are edible as namul, soup and fries [11]. Gondre-namul-bap is a common type of namul that is made with dried gondre, seasoned with perilla oil, and served over rice as a local cuisine in Kangwon Jeongseon [12]. In this process, boiled gondre is used for namul manufacture, with boiled water produced as byproducts.

C. setidens has been used as a Korean traditional medicine to treat hemostasis, hematoma, hematuria and hypertension [13]. Pectolinarin has been reported as a major compound in *Cirsium* species such as *C. setidens* and has various biological activities, including antioxidant, anti-melanogenesis, anti-tumor, anti-inflammatory, anti-cancer, and hepato-protective effects [14–20].

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C. setidens and its isolate scopoletin have been reported to inhibit melanin biosynthesis in B16F10 cells [21,22]. However, many studies have identified the major compounds from *C. setidens* as pectolinarin and its derivative pectolinarigenin [20,23–26]. However, the effects of pectolinarin and pectolinarigenin on melanogenesis have not been reported. Pectolinarin was isolated as a primary compound with hepatoprotective activity and was then converted into pectolinarigenin via acid hydrolysis. Both pectolinarin and pectolinarigenin have protective effects against GalN-induced hepatic injury via antioxidant activity and an anti-inflammatory decrease of eicosanoid formation [18,19,27]. Byproducts of Gondre namul may be useful as melanogenesis inhibitors because boiled Gondre water is a rich source of water soluble secondary metabolites from *C. setidens*. In our present study, we investigated the inhibitory effect of pectolinarin and pectolinarigenin on melanogenesis and an efficient component conversion method to convert pectolinarin from *C. setidens* into its pectolinarigenin aglycone.

2. Materials and methods

2.1. Cell culture

Melan-a cells were obtained from Dr. Dorothy C. Bennett (University of London, London, UK) and grown in RPMI 1640 medium (Roswell Park Memorial Institute 1640; HyClone, Logan, UT, USA) supplemented with 10% FBS (fetal bovine serum; HyClone), 100 U/ml penicillin and 100 μg/ml streptomycin (HyClone) and 200 nM TPA (tetradecanoylphorbol 13-acetate; Sigma-Aldrich Co. St. Louis, MO, USA) at 37 °C in a humidified incubator with a 5% CO_2/95% air atmosphere. Cells were sub-cultured every 3 days until a maximum passage of 40 was reached.

2.2. Cell viability assay

Cell viability was tested by the MTT assay. Cells were seeded in a 96-well plate (2×10^4 cells/well) for 24 h, washed with PBS (Phosphate Buffered Saline; Welgene, Gyeongsan, Korea), and treated with or without pectolinarin (30 μM) or pectolinarigenin (30 μM). After 72 h of incubation, the MTT reagent was added to each well, and the plate was incubated at 37 °C for 2 h. The medium was discarded, and the plate was washed with PBS. The intracellular formazan was dissolved in DMSO (Dimethyl sulfoxide; Sigma-Aldrich Co.) and absorbance measured at 595 nm using a microplate reader (BIO-TEK Power Wave XS, Winooski, VT, USA).

2.3. Melanin content measurements

The melan content of melan-a cells were measured as described previously [28]. Briefly, melan-a cells were seeded in 24-well plates (1×10^5 cells/well), incubated for 24 h, washed with PBS, and treated with or without pectolinarin (30 μM) or pectolinarigenin (30 μM). After 72 h, cells were washed with PBS and lysed with 2 N NaOH. The lysed cells were transferred into 96-well plates, and absorbance was measured at 475 nm using a microplate reader (BIO-TEK Power Wave XS) to determine the melanin content.

2.4. Measurement of intracellular tyrosinase activity

Cells were seeded in a 60-mm dish (4×10^5 cells/dish) for 24 h, washed with PBS (Welgene), and treated with or without pectolinarin (30 μM) or pectolinarigenin (30 μM). After 72 h of incubation, the cells were washed with PBS and lysed in 1% Triton X-100. Then, the lysed cells were chilled on ice for 10 min and centrifuged, and supernatant was collected to determine the enzyme source of the tyrosinase assay. The reaction mixture contains 100 μL of 0.1 M phosphate buffer (pH 6.5), 100 μL of 20 mM L-DOPA and 40 μg of cell lysates in each well of a 96-well microplate. The initial absorbance was measured at 490 nm using a BIO-TEK Power Wave XS microplate reader, and the reaction mixture was incubated at room temperature. After 1 h, the final absorbance was measured at the same wavelength. Intracellular tyrosinase activity was estimated the ratio to control.

2.5. Western blotting analysis

For protein expression analysis, melan-a cells were harvested and homogenized at 4 °C in lysis buffer. After centrifugation, cell debris was discarded, and the protein concentrations were determined using the BCA (bicinchoninic acid) assay. Twenty μg of protein was separated in 10% SDS–PAGE gels and then transferred to PVDF membranes (polyvinylidene fluoride membrane, Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk at room temperature for 2 h and then incubated with primary antibodies. Anti-Tyrosinase, anti-TRP-1, anti-TRP-2 (Santa Cruz, CA, USA) and anti-MITF (Microphthalmia-associated transcription factor, Cell signaling Technology, Beverly, MA, USA) for western blotting were used as primary antibodies. Anti-goat IgG-horseradish peroxidase (HRP) and anti-mouse IgG-HRP were purchased from Santa Cruz and used as secondary antibodies. The reaction was progressed using a SuperSignal® West Femto Maximum Sensitivity Substrate (Pierce, Rockford, IL, USA). Immuno-reactive bands were visualized using an enhanced LAS 4000 film (Fuji film, Tokyo, Japan). An anti-GAPDH antibody was used to monitor protein loading in each lane. Densitometric analysis was performed using the ImageJ software.

2.6. Quantitative real-time PCR (Q-PCR) analysis of mRNA expression

Total RNA was extracted using an RNeasy mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocols and stored at -80 °C until use. cDNA was amplified using a ImProm-II Reverse Transcription System (Promega, Madison, WI, USA) according to the manufacturer's guidelines. Then, SYBR green-based quantitative PCR was performed using the Applied Biosystems 7500 Fast Real-Time PCR system and Fast SYBR® Green Master Mix (Life technologies, UK). All reactions were run in triplicate, and the data were analyzed using the $2^{-\Delta\Delta C}{}_T$ values method [29]. The sequences of the primers used in this study were MITF forward: 5′-ATG GAC GAC ACC CTT TCT C-3’; MITF reverse: 5′-GGA GGA TTC GCT AAC AAG TG-3’; Tyrosinase forward: 5′-GGC CAG CTT TCA GGC AGA GGT-3’; Tyrosinase reverse: 5′-TGG TGC TTC ATG GGC AAA ATC-3’; TRP1 forward: 5′-AAG CAG ACA TCC AAC AAC ACT AG-3’; TRP1 reverse: 5′-GCA AGA GTT CAG AAC ACA GGT C-3’; TRP2 forward: 5′-GCA AGA GAT ACA CGG AGG AAG-3’; TRP2 reverse: 5′-CTA AGG CAT CAT CAT CAT CAC TAC-3’; β-ctin forward: 5′-GAC AGG ATG CAG AAG GAG ATT ACT-3’; β-actin reverse: 5′- TGA TCC ACA TCT GCT GGA AGG T-3’. The quantity of each transcript was calculated as described in the instrument manual and normalized to the amount of β-actin.

2.7. Evaluation of inhibitory efficacy of PG on the reconstructed skin model

A reconstructed human skin model (Neoderm®-ME; Tego Science, Seoul, Korea) consisting of human epidermal melanocytes and human-derived epidermal keratinocytes was incubated in serum-free maintenance medium (Tego Science). The reconstructed human skin was incubated in the presence of

pectolinarigenin (30 μM) for 2 days. Reconstructed human epidermis was fixed in 10% formalin, blocked in paraffin and stained for melanin using L-DOPA and the Fontana-Masson staining method.

2.8. Plant material and extraction

The dried aerial parts of *Cirsium setidens* (Dunn) Nakai were purchased at Jeongseon Herb market (Jeongseon, Korea) in June 2016 and identified by Prof. Eun Ju Jeong of the Department of Agronomy and Medicinal Plant Resources, Gyeongnam University of Science and Technology. A voucher specimen (SN20160611) has been deposited at the KIST Gangneung Institute of natural products. Dried aerial parts (100 g) of *C. setidens* were extracted thrice with water (1 L) under heating for 3 h. The hot-water extract was concentrated under reduced pressure to yield a water extract (13.7 g) for further study.

2.9. Hydrolysis of hot-water C. setidens extract

The microwave system used was a Microwave Digestion System, Model MDS 2000 (CEM Corporation, Seoul, Korea). The unit offers a wide range of hydrolysis conditions and can operate at 150–205 °C (power: 100 W, 2455 MHz). Each *C. setidens* extract dissolved in water or 1% acetic acid (2 mL) was poured into a PTFE reactor, sealed and heated at 100 W. The reactor was allowed to cool between each heating and finally cooled to room temperature.

2.10. LC/MS analyses

Test samples were analyzed for pectolinarin and pectolinarigenin using the LC/MS method reported previously.[19] The LC/MS system consisted of an Agilent 1200 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA), equipped with ESI mode. Test samples and standard compounds were dissolved in MeOH using

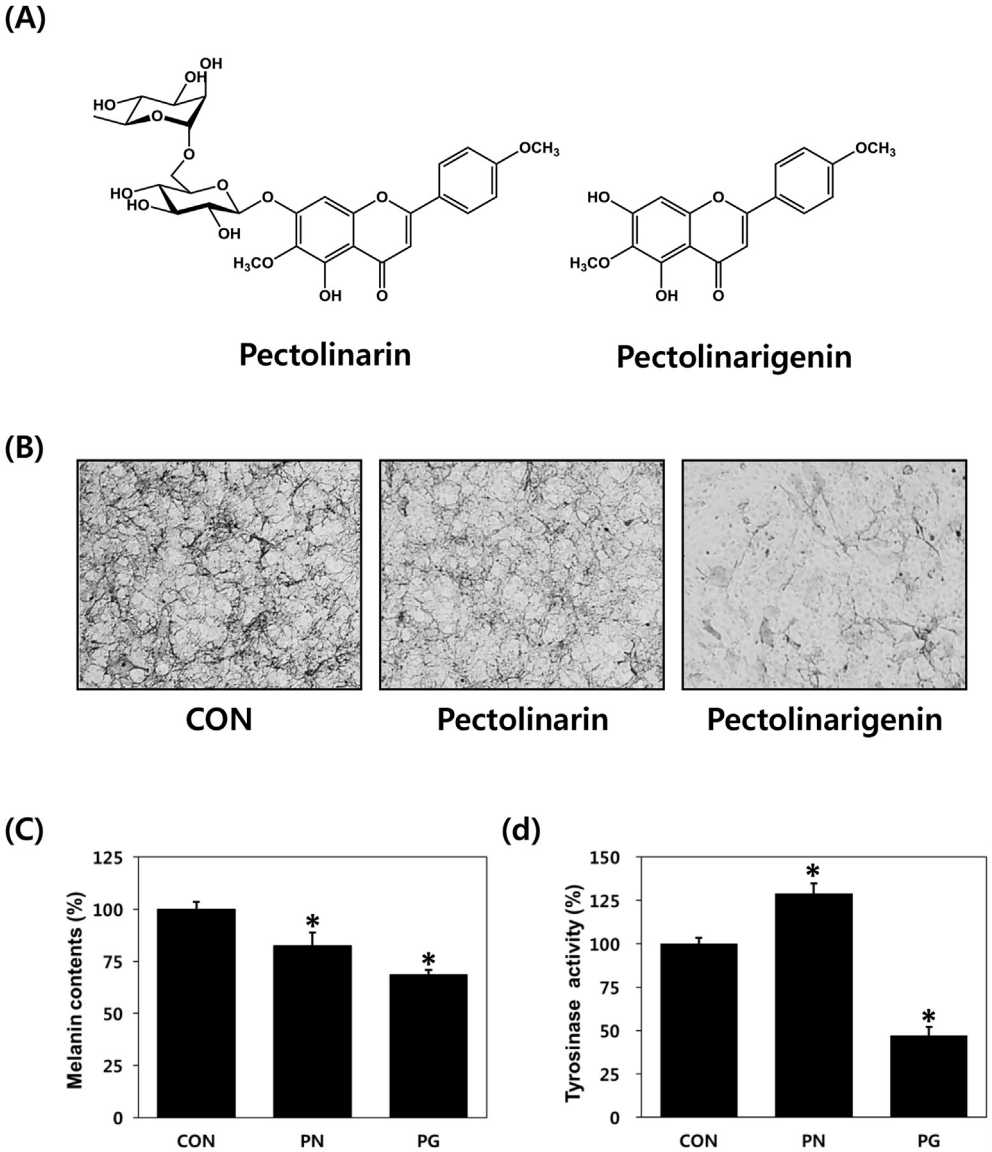

Fig. 1. Inhibitory effects of pectolinarin and pectolinarigenin on melanin synthesis in melan-a cells. (A) Chemical structures of pectolinarin (PN) and pectolinarigenin (PG). Melanin content (B) and intracellular tyrosinase activity (C) in melan-a cells incubated with 30 μM of PN and PG. Each bar represents the mean ± S.D. of triplicate measurements. *P < 0.05 vs. control (CON).

an ultrasonicator and a vortex mixer and were then filtered through a 0.45 μm syringe filter before injection. The mobile phase was a mixed solvent of 0.05% trifluoroacetic acid (TFA) in acetonitrile (solvent A) and 0.05% TFA in water (solvent B). The gradient elution system was as follows: (A)/(B) = 10/90 (0 min; hold for 3 min) → 90/10 (13 min) →90/10 (13 min; hold for 5 min) → 95/5 (20 min). Column temperature was maintained at 40 °C using a temperature controller. Analysis was performed at a flow rate of 1.00 mL/min, with the detection wavelength fixed at 340 nm.

2.11. Statistical analysis

Data were analyzed for statistical significance using Student's *t*-test. *P* values of <0.05 were considered to indicate statistically significant differences. The mean, SD and SEM were calculated for all variables.

3. Results

3.1. Inhibitory effects of pectolinarin and pectolinarigenin on melanin biosynthesis in melan-a cells

To study the effects of pectolinarin and pectolinarigenin (Fig. 1A) on melanin biosynthesis, after treatment with pectolinarin and pectolinarigenin at 30 μM for 72 h, the melanin content and viability were measured. Neither pectolinarin nor pectolinarigenin showed cytotoxicity at indicated concentrations. Treating melan-a cells with pectolinarin (30 μM) or pectolinarigenin (30 μM) reduced the melanin content to 17.4 ± 6.2% and 31.5 ± 2.2%, respectively, compared with controls (Fig. 1B and C). These results showed that pectolinarigenin suppressed melanin biosynthesis without cytotoxicity to a significantly higher degree than did pectolinarin treatment of melan-a cells. Reduced tyrosinase activity in melan-a cells could be produced either by the repression of tyrosinase gene expression or by the direct inhibition of tyrosinase. Cell lysates treated with pectolinarin and pectolinarigenin were utilized as tyrosinase sources. Intracellular tyrosinase activity was suppressed by pectolinarigenin treatment, but pectolinarin did not inhibit intracellular tyrosinase activity in melan-a cells. Treating melan-a cells with pectolinarigenin (30 μM) decreased the intracellular tyrosinase activity to 46.9 ± 4.5% of the control group (Fig. 1D).

3.2. The effects of pectolinarin and pectolinarigenin on melanin biosynthesis-related proteins and gene expression in melan-a cells

We investigated the effect that pectolinarin and pectolinarigenin had on expression of melanin biosynthesis-related proteins, MITF, tyrosinase, TRP1, and TRP2, by western blotting using cell lysates treated with pectolinarin and pectolinarigenin. Pectolinarigenin reduced the protein expression of Tyrosinase, TRP1, and TRP2 by 37.9 ± 3.2%, 42.0 ± 2.6%, and 38.3 ± 3.8%, respectively, compared with the control group (Fig. 2A and B). MITF is the most important transcription factor that regulates the expression of melanin biosynthesis-related genes [30]. Pectolinarigenin dramatically suppressed MITF protein expression by 51.5 ± 8.5% in melan-a cells. It has been suggested that the down-regulation of melanin biosynthesis-related proteins by pectolinarigenin might be related to MITF signaling. To investigate whether the inhibition of protein expression was associated with reduced the mRNA levels of melanin biosynthesis-related genes, the mRNA expression levels of Tyrosinase, TRP-1, TRP-2, and MITF were confirmed using Q-PCR. Pectolinarigenin reduced the gene expression of Tyrosinase, TRP-1 and MITF by 55.0 ± 6.1%, 10.7 ± 3.3%, and 32.3 ± 1.0%, respectively (Fig. 2C). These results indicate that the presence of pectolinarigenin suppressed the expression of tyrosinase-related genes, suggesting a mechanism of down-regulated MITF transcription.

3.3. Effect of melanin biosynthesis by pectolinarigenin in a reconstructed human skin model

To approximate human usage, a reconstructed human skin model was used to identify the depigmenting effect of pectolinarigenin. The melanin content was significantly reduced in pectolinarigenin-treated reconstructed human skin compared with the control group according to visual and spectrophotometric evaluations (Fig. 3A). Treating reconstructed human skin with pectolinarigenin decreased the melanin content to 20.8% of the control group (Fig. 3B). Additionally, treating reconstructed human skin with pectolinarigenin decreased the L-DOPA content compared with the control group (Fig. 3C). These results imply pectolinarigenin is an anti-melanogenic material and has useful applications in human skin.

3.4. Conversion of pectolinarin to pectolinarigenin

For the simultaneous determination of pectolinarin and pectolinarigenin in *C. setidens* hot-water extract, the optimized chromatographic conditions were investigated. Peaks of 2 compounds in the chromatograms were determined by comparing on-line UV spectra and the retention times with those of the standards. Retention time for pectolinarin and pectolinarigenin of *C. setidens* hot-water extract were 10.33 and 13.57 min, respectively (Fig. 4A). *C. setidens* hot-water extract with microwave hydrolysis (205 °C) increased pectolinarigenin contents 66.3% compared with that of the control group. In comparison, pectolinarin content was decreased to 14.5% (Fig. 4B). These results indicate that microwave irradiation efficiently converted the pectolinarin into pectolinarigenin. Additionally, this reaction occurred more efficiently under the 1% acetic acid conditions than in neutral solution. These results suggest that microwave irradiation under 1% acetic acid conditions is optimal to convert pectolinarin of *C. setidens* hot-water extract into pectolinarigenin.

4. Discussion

Cirsium setidens, gondre, used as a type of namul made with dried gondre, is a favored local cuisine in Kangwon Jeongseon. In this process, boiled gondre is used for namul manufacture, with boiled water produced as byproducts. However, no previous study has examined the anti-melanogenic activity and byproduct utilization of *C. setidens*. Among the constituents of *C. setidens*, pectolinarin was isolated as a primary compound possessing several activities, and it was converted into pectolinarigenin via acid hydrolysis [18,19,27]. Through a preliminary study, we found that pectolinarin were recrystallized from gondre-boiled water byproducts. In this study, we investigated anti-melanogenesis effects of pectolinarin from *C. setidens* and its aglycone pectolinarigenin in melanocytes and a reconstituted skin model. Furthermore, to increase the active component content in *C. setidens* water extracts, several component conversion methods were studied.

We performed *in vitro* tests to investigate anti-melanogenic effects by pectolinarin and pectolinarigenin. Both pectolinarin and pectolinarigenin inhibited melanin synthesis without cytotoxicity in melan-a cells. Pectolinarigenin treatment showed more potent inhibitory activity of melanin synthesis than did pectolinarin treatment. In agreement with the observed effects on melanin content, the intracellular tyrosinase activity was also significantly reduced by pectolinarigenin. However, treatment with pectolinarin increased the intracellular tyrosinase activity. As a result,

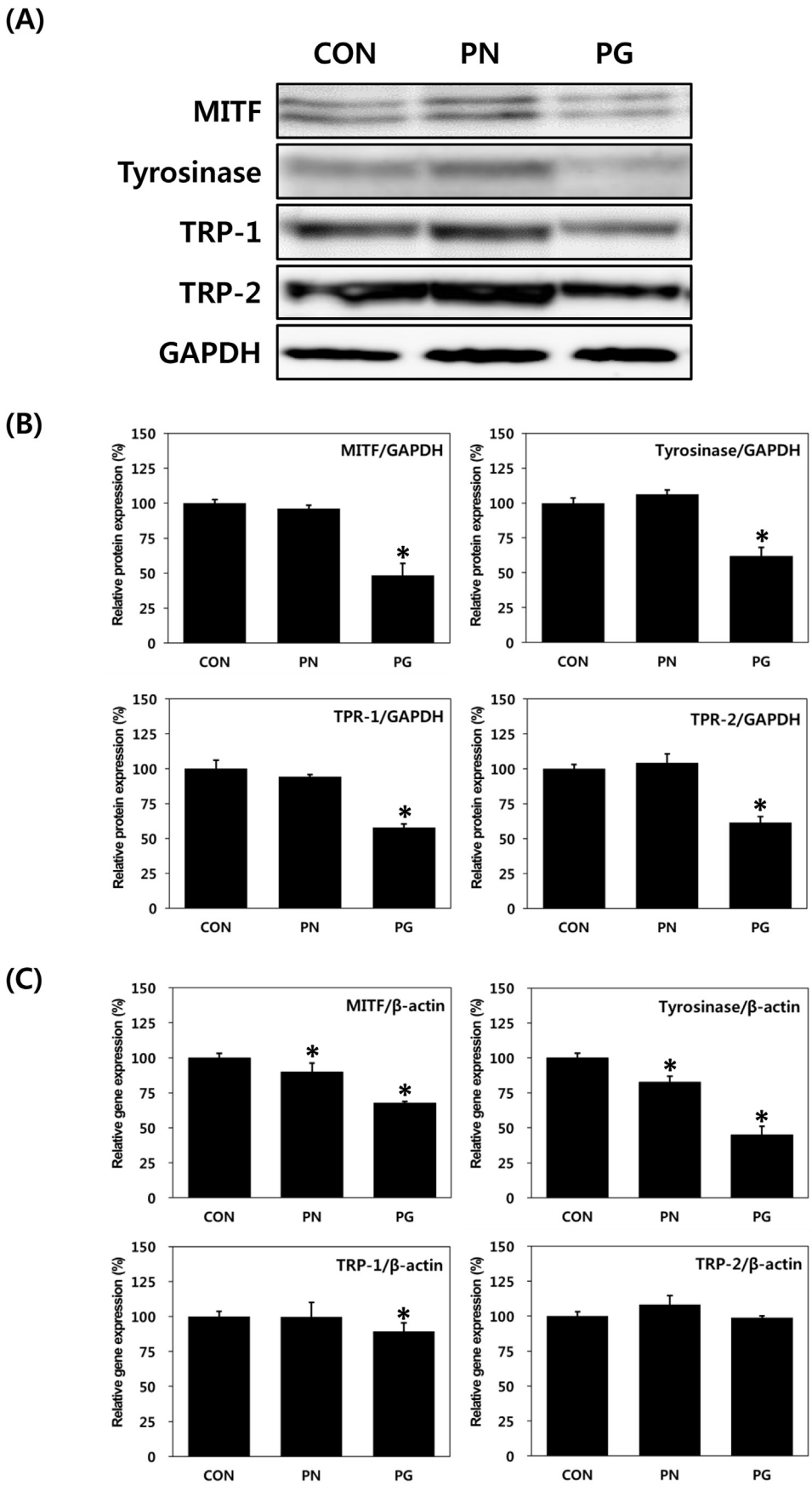

Fig. 2. Inhibitory effects of pectolinarin and pectolinarigenin on expression of melanogenesis-related protein and mRNA. (A and B) The protein expression levels of MITF, tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2) were determined by western blot analysis and normalized to GAPDH expression. Each bar represents the mean ± S.D. of triplicate measurements. *P < 0.05 vs. control (CON). (C) Relative gene expression of MITF, tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2) were examined by Q-PCR and normalized to β-actin. Each bar represents the mean ± S.D. of triplicate measurements. *P < 0.05 vs. control (CON).

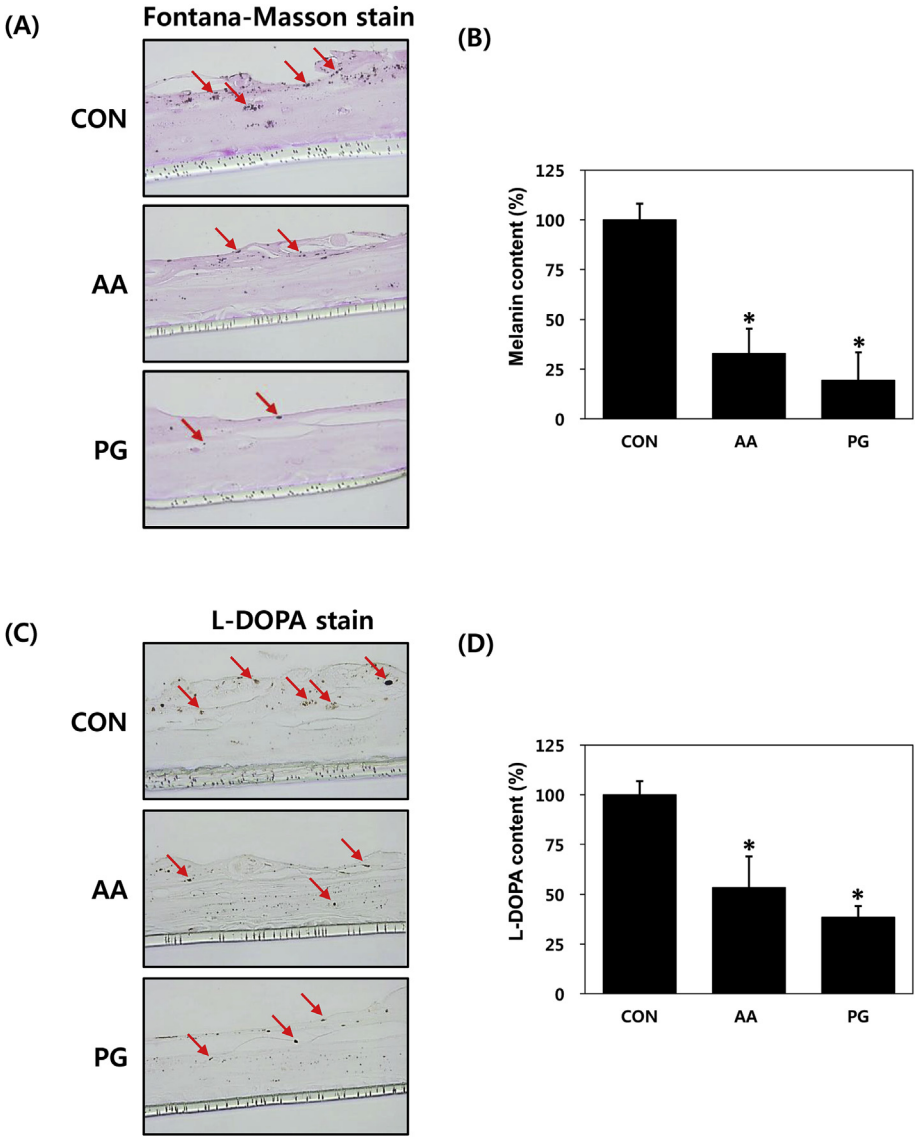

Fig. 3. The inhibitory effect of pectolinarigenin on melanin production in a reconstructed human skin model. (A) Histopathological features of Pigmentation and L-DOPA in reconstructed skin. The sections were stained with Fontana-Masson (FM) and L-DOPA (magnification, 100 ×). Melanin (B) and L-DOPA (C) contents were determined using ImageJ software. The results are expressed as the mean ± S.D. of three randomly selected sites. *P < 0.05 vs. control (CON). AA: Ascorbic acid, PG: pectolinarigenin.

pectolinarigenin was effective at inhibiting the signs related to melanogenesis in melan-a cells.

We studied the effects that pectolinarin and pectolinarigenin had on the expression of melanin biosynthesis-related proteins MITF, Tyrosinase, TRP-1, and TRP-2, by western blotting. Only pectolinarigenin inhibited the protein expression of MITF, tyrosinase, TRP-1, and TRP-2. MITF is a key transcription factor that regulates the expression of most melanogenesis-related genes [30]. Pectolinarigenin dramatically suppressed MITF mRNA and protein expression in melan-a cells. This finding suggests that pectolinarigenin could be associated with altered MITF signaling to down-regulate melanogenesis-related proteins. However, treatment with pectolinarin did not suppress melanogenesis-related proteins and genes. There are significant differences between the anti-melanogenic effects of the two compounds.

To investigate the depigmenting activity of pectolinarigenin in a human skin-like system, we used Neoderm®-ME, a reconstructed human skin model. In agreement with the inhibition of melanogenesis in cells, the melanin and L-DOPA contents in reconstructed human skin were decreased by pectolinarigenin. These results suggest the possibility of using pectolinarigenin as an anti-melanogenic agent that is applicable in human skin. Based on these and previous results, we confirmed that pectolinarigenin, unlike pectolinarin, may be an effective approach for developing anti-melanogenesis therapeutics.

The concentrations of pectolinarin and pectolinarigenin were determined in *C. setidens* water extract by HPLC. Unfortunately, the amount of pectolinarigenin of *C. setidens* water extract was lower than that of pectolinarin. To increase the pectolinarigenin content in *C. setidens* water extract, several component conversion methods were studied. *C. setidens* hot-water extract with high power microwave irradiation increased the pectolinarigenin content and decreased the pectolinarin content. These results indicate that microwave irradiation efficiently converted the pectolinarin into pectolinarigenin. Additionally, this reaction occurred more efficiently under the 1% acetic acid conditions than in aqueous

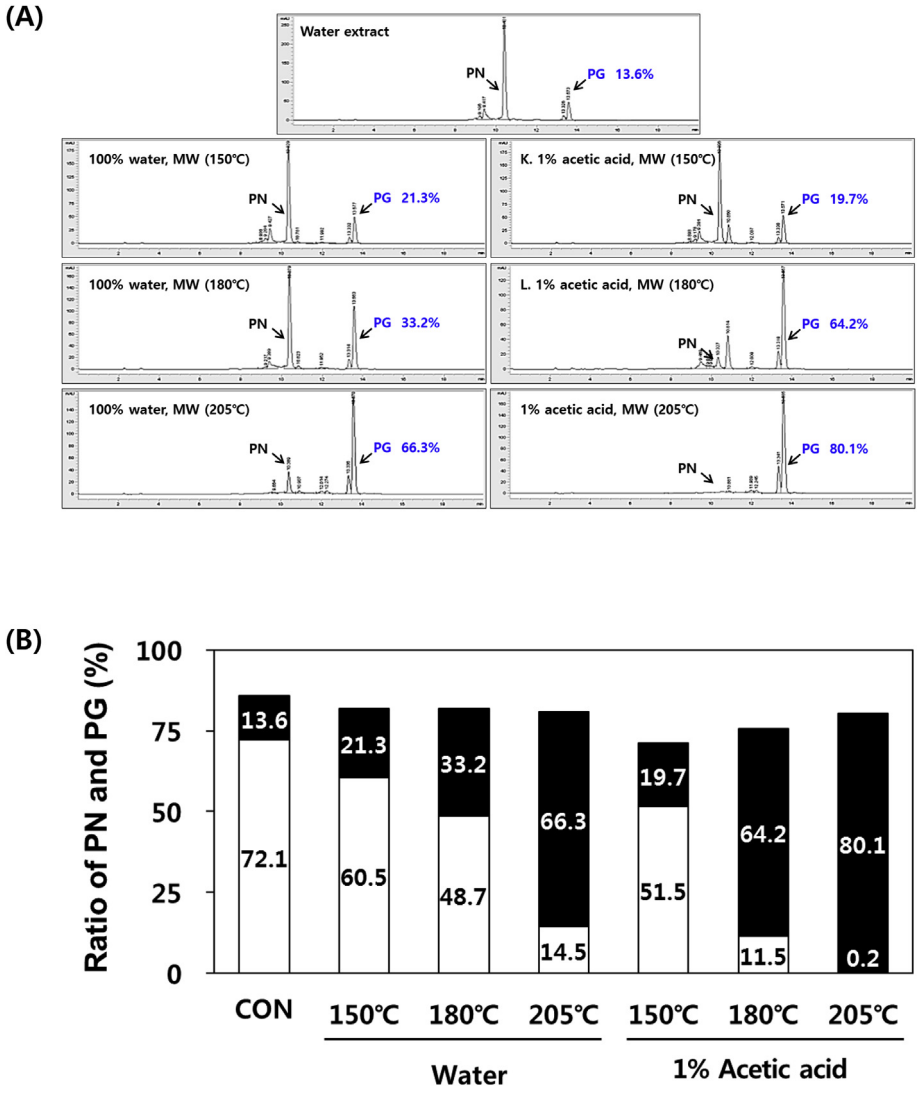

Fig. 4. The conversion effect of pectolinarin to pectolinarigenin by microwave irradiation. (A) HPLC chromatogram of *C. setidens* hot-water extract and microwave irradiated extract. The presence of PN (t_R 10.33 min) and PG (t_R 13.57 min), in extracts were verified by comparing each retention time and UV spectrum. (B) The efficiency of conversion to PG by microwave irradiation and under acidic conditions.

solution. These results suggest that microwave irradiation under 1% acetic acid conditions converts pectolinarin from *C. setidens* hot-water extract into pectolinarigenin. Consequently, we identified that microwave irradiation under 1% acetic acid was the optimum sugar elimination method. These results indicate that pectolinarin could be isolated from gondre byproducts as a primary compound used for anti-melanogenesis activity and then converted into pectolinarigenin via microwave irradiation.

In conclusion, pectolinarigenin treatment showed more potent inhibitory activity of melanin synthesis than did pectolinarin *in vitro*. There are significant differences in the anti-melanogenesis effects of the two compounds. Pectolinarigenin inhibited the melanogenesis without toxicity *in vivo*. Additionally, pectolinarigenin decreased the melanin content and L-DOPA in reconstructed human skin. Based on the results obtained for pectolinarin and pectolinarigenin, we confirmed that only pectolinarigenin may be an effective approach for anti-melanogenesis. Pectolinarin could be isolated from gondre byproducts as a primary compound used for anti-melanogenesis activity and then converted into pectolinarigenin via microwave irradiation.

Conflict of interest

The authors declare no competing financial interests.

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References

[1] R.R. Gates, A.A. Zimmermann, Comparison of skin color with melanin content1, J. Investigative Dermatology 21 (1953) 339–348.
[2] C.J. Cooksey, P.J. Garratt, E.J. Land, S. Pavel, C.A. Ramsden, P.A. Riley, N.P. Smit,

Evidence of the indirect formation of the catecholic intermediate substrate responsible for the autoactivation kinetics of tyrosinase, J. Biol. Chem. 272 (1997) 26226–26235.
[3] E. Kvam, R.M. Tyrrell, The role of melanin in the induction of oxidative DNA base damage by ultraviolet A irradiation of DNA or melanoma cells, J. Investigative Dermatology 113 (1999) 209–213.
[4] Y. Miyamura, S.G. Coelho, R. Wolber, S.A. Miller, K. Wakamatsu, B.Z. Zmudzka, S. Ito, C. Smuda, T. Passeron, W. Choi, Regulation of human skin pigmentation and responses to ultraviolet radiation, Pigment Cell Res. 20 (2007) 2–13.
[5] V.J. Hearing, Determination of melanin synthetic pathways, J. Investigative Dermatology 131 (2011) E8–E11.
[6] J.Y. Lin, D.E. Fisher, Melanocyte biology and skin pigmentation, Nature 445 (2007) 843–850.
[7] N.L. Lacz, J. Vafaie, N.I. Kihiczak, R.A. Schwartz, Postinflammatory hyperpigmentation: a common but troubling condition, Int. J. dermatology 43 (2004) 362–365.
[8] A.A. Brożyna, L. VanMiddlesworth, A.T. Slominski, Inhibition of melanogenesis as a radiation sensitizer for melanoma therapy, Int. J. cancer 123 (2008) 1448–1456.
[9] H. Ando, H. Kondoh, M. Ichihashi, V.J. Hearing, Approaches to identify inhibitors of melanin biosynthesis via the quality control of tyrosinase, J. Investigative Dermatology 127 (2007) 751–761.
[10] I. Kang, C. Chung, S. Ham, S. Lee, D. Oh, K. Choi, J. Do, Development of fermented soysauce using Cirsium setidens Nakai and comfrey, J. Korean Soc. Food Sci. Nutr. (Korea Repub). 6 (1997) 1152–1158.
[11] S.-H. Lee, Y.-S. Jin, S.-I. Heo, T.-H. Shim, J.-H. Sa, D.-S. Choi, M.-H. Wang, Composition analysis and antioxidative activity from different organs of cirsium setidens nakai, Korean J. Food Sci. Technol. 38 (2006) 571–576.
[12] S.Y. Chang, J.H. Song, Y.S. Kwak, M.J. Han, Quality characteristics of Gondre tofu by the level of Cirsium setidens powder and storage, J. Korean Soc. Food Cult. 27 (2012) 737–742.
[13] S. Lee, Korean Folk Medicine-monographs Series. No. 3, Publishing Center of Seoul National University, Seoul, Korea, 1966, p. 88.
[14] J.-H. Roh, O.-P. Zee, H.-I. Moon, Phytochemical constituents from melampyrum roseum var. hirsutum beauv, Korean J. Pharmacogn. 31 (2000).
[15] J.-C. Do, K.-Y. Jung, K.-H. Son, Isolation of pectolinarin from the aerial parts of Cirsium nipponicum, Korean J. Pharmacogn. 25 (1994).
[16] S. Liu, J. Zhang, D. Li, W. Liu, X. Luo, R. Zhang, L. Li, J. Zhao, Anticancer activity and quantitative analysis of flavone of Cirsium japonicum DC, Nat. Prod. Res. 21 (2007) 915–922.
[17] S. Liu, X. Luo, D. Li, J. Zhang, D. Qiu, W. Liu, L. She, Z. Yang, Tumor inhibition and improved immunity in mice treated with flavone from cirsium japonicum DC, Int. Immunopharmacol. 6 (2006) 1387–1393.
[18] R. Tundis, B. Deguin, M.R. Loizzo, M. Bonesi, G.A. Statti, F. Tillequin, F. Menichini, Potential antitumor agents: flavones and their derivatives from linaria reflexa desf, Bioorg. Med. Chem. Lett. 15 (2005) 4757–4760.
[19] M. Martínez-Vázquez, T.O.R. Apan, A.L. Lastra, R. Bye, A comparative study of the analgesic and anti-inflammatory activities of pectolinarin isolated from Cirsium subcoriaceum and linarin isolated from Buddleia cordata, Planta medica 64 (1998) 134–137.
[20] H. Lim, K.H. Son, H.W. Chang, K. Bae, S.S. Kang, H.P. Kim, Anti-inflammatory activity of pectolinarigenin and pectolinarin isolated from Cirsium chanroenicum, Biol. Pharm. Bull. 31 (2008) 2063–2067.
[21] S.J. Hur, E.Y. Park, M.J. Ann, A.R. Jang, K.A. Yang, W.K. whang, A study on the whitening effects and HPLC pattern analysis of cirsium setidens nakai, Korean J. Aesthetic Soc. 8 (2010) 1–9.
[22] M.-J. Ahn, S.-J. Hur, E.-H. Kim, S.H. Lee, J.S. Shin, M.-K. Kim, J.A. Uchizono, W.-K. Whang, D.-S. Kim, Scopoletin from Cirsium setidens increases melanin synthesis via CREB phosphorylation in B16F10 cells, Korean J. Physiology Pharmacol. 18 (2014) 307–311.
[23] H.C. Jeong, Y.-S. Shim, Y.K. Rhee, S.Y. Choi, H.-D. Hong, J. Chung, M.J. Han, C.-W. Cho, Quantification of marker compounds in cirsium setidens nakai by HPLC-DAD, Food Sci. Biotechnol. 22 (2013) 1481–1486.
[24] A. Nugroho, S.C. Lim, J.S. Choi, Articles: validation of high-performance liquid chromatography analysis on phenolic substances of Cirsium setidens and sedative effect of pectolinarin as the active principle, Nat. Product. Sci. 17 (2011) 342–349.
[25] J.-W. Oh, J.-H. Lee, M.-L. Cho, G.-H. Shin, J.-M. Kim, S.-I. Choi, T.-D. Jung, Y.-H. Kim, S.-J. Lee, B.J. Lee, Development and validation of analytical method for pectolinarin and pectolinarigenin in fermented Cirsium setidens Nakai by bioconversion, J. Korean Soc. Food Sci. Nutr. 44 (2015) 1504–1509.
[26] J.H. Lee, H.K. Jung, Y.S. Han, Y.M. Yoon, C.W. Yun, H.Y. Sun, H.W. Cho, S.H. Lee, Antioxidant effects of Cirsium setidens extract on oxidative stress in human mesenchymal stem cells, Mol. Med. Rep. 14 (2016) 3777–3784.
[27] Y.-M. Yoo, J.-H. Nam, M.-Y. Kim, J. Choi, H.-J. Park, Pectolinarin and pectolinarigenin of Cirsium setidens prevent the hepatic injury in rats caused by D-galactosamine via an antioxidant mechanism, Biol. Pharm. Bull. 31 (2008) 760–764.
[28] S.-H. Baek, I.-J. Nam, H.S. Kwak, K.-C. Kim, S.-H. Lee, Cellular anti-melanogenic effects of a euryale ferox seed extract ethyl acetate fraction via the lysosomal degradation machinery, Int. J. Mol. Sci. 16 (2015) 9217–9235.
[29] K.J. Livak, T.D. Schmittgen, Analysis of Relative Gene Expression Data Using Real-time Quantitative PCR and the 2− ΔΔCT Method, Methods, vol. 25, 2001, pp. 402–408.
[30] W. Xu, L. Gong, M.M. Haddad, O. Bischof, J. Campisi, E.T. Yeh, E.E. Medrano, Regulation of microphthalmia-associated transcription factor MITF protein levels by association with the ubiquitin-conjugating enzyme hUBC9, Exp. Cell Res. 255 (2000) 135–143.