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

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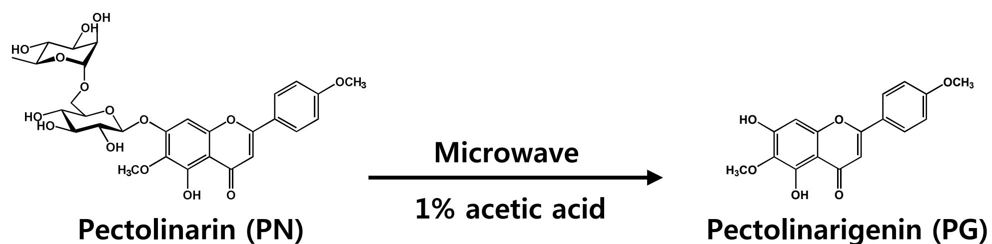
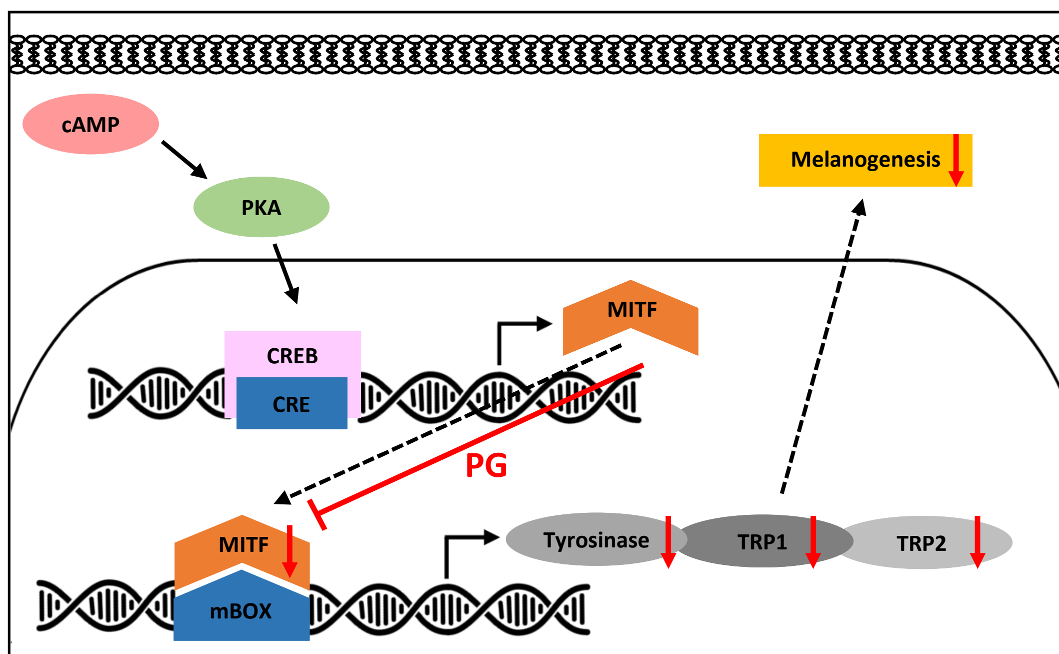


Microwave
1% acetic acid
Pectolinarin (PN)
Pectolinarigenin (PG)



cAMP
PKA
Melanogenesis
CREB
CRE
MITF
PG
MITF
mBOX
Tyrosinase
TRP1
TRP2

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1 **Pectolinarigenin, an aglycone of pectolinarin, has more potent inhibitory**

2 **activities on melanogenesis than pectolinarin**

3

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13 **Running title:** Pectolinarigenin has potent whitening effects

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

37 **Keywords:** Whitening, Melanogenesis, Cirsium setidens, Microwave, Pectolinarin,
38 Pectolinarigenin

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41 ## 1. Introduction

42 Melanin is a natural pigment that is produced by a specialized group of cells known as
43 melanocytes and melanoma cells and by the oxidation of the amino acid tyrosine followed by
44 polymerization. It plays a key role in protecting cells from cytotoxic light and determines the
45 skin color of mammals.[1,2,3] In the skin, melanin is synthesized to defend against UV
46 damage through a complex process that involves various enzymes and a series of signal
47 pathways.[4] The biosynthesis of melanin, called melanogenesis, begins with the oxidation of
48 tyrosinase to DQ (DOPAquinone) via the intermediate DOPA (3,4-dihydroxyphenylalanine).[5]
49 Second, TRP-2 (tyrosinase-related protein 2) converts DQ into DOPAchrome and
50 subsequently converts DOPAchrome into DHICA (indole 5,6-quinone 2-carboxylic acid) or DHI
51 (5,6-dihydroxyindole). Finally, TRP-1 (tyrosinase-related protein 1) converts DHICA and forms
52 eumelanins.[6] However, the overproduction of melanin causes medical problems such as
53 melanoderma, post-inflammatory diseases and melasma.[7,8] Since the development of
54 anti-melanogenic agents is an important goal in the clinical and cosmetic fields, many studies
55 have focused on understanding the exact mechanism of melanogenesis. Traditionally,
56 researchers tried to develop anti-melanogenic agents and have focused on small molecules
57 or natural products that serve as inhibitors of tyrosinase, the rate-limiting enzyme in
58 melanogenesis.[9]

59 *Cirsium setidens* Nakai is a perennial plant of the aster family in the genus *Cirsium*. *C.*
60 *setidens*, also known as “gondre” in English, is found mainly in the Kangwon province of
61 Korea.[10] The young leaves and stems of *C. setidens* are rich in protein, calcium, and vitamin
62 A and are edible as namul, soup and fries.[11] Gondre-namul-bap is a common type of
63 namul that is made with dried gondre, seasoned with perilla oil, and served over rice as a

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64 local cuisine in Kangwon Jeongseon.[12] In this process, boiled gondre is used for namul
65 manufacture, with boiled water produced as byproducts.

66 *C. setidens* has been used as a Korean traditional medicine to treat hemostasis, hematoma,
67 hematuria and hypertension.[13] Pectolinarin has been reported as a major compound in
68 *Cirsium* species such as *C. setidens* and has various biological activities, including antioxidant,
69 anti-melanogenesis, anti-tumor, anti-inflammatory, anti-cancer, and hepato-protective
70 effects.[14,15,16,17,18,19,20] *C. setidens* and its isolate scopoletin have been reported to
71 inhibit melanin biosynthesis in B16F10 cells.[21,22] However, many studies have identified
72 the major compounds from *C. setidens* as pectolinarin and its derivative pectolinarigenin.
73 [20,23,24,25,26] However, the effects of pectolinarin and pectolinarigenin on melanogenesis
74 have not been reported. Pectolinarin was isolated as a primary compound with
75 hepatoprotective activity and was then converted into pectolinarigenin via acid hydrolysis.
76 Both pectolinarin and pectolinarigenin have protective effects against GalN-induced hepatic
77 injury via antioxidant activity and an anti-inflammatory decrease of eicosanoid formation.
78 [18,19,27] Byproducts of Gondre namul may be useful as melanogenesis inhibitors because
79 boiled Gondre water is a rich source of water soluble secondary metabolites from *C. setidens*.
80 In our present study, we investigated the inhibitory effect of pectolinarin and
81 pectolinarigenin on melanogenesis and an efficient component conversion method to
82 convert pectolinarin from *C. setidens* into its pectolinarigenin aglycone.

83

84 **2. Materials and Methods**

85 *2.1. Cell Culture*

86 Melan-a cells were obtained from Dr. Dorothy C. Bennett (University of London, London,

87 UK) and grown in RPMI 1640 medium (Roswell Park Memorial Institute 1640; HyClone, Logan,
88 UT, USA) supplemented with 10% FBS (fetal bovine serum; HyClone), 100 U/ml penicillin and
89 100 μg/ml streptomycin (HyClone) and 200 nM TPA (tetradecanoylphorbol 13-acetate;
90 Sigma-Aldrich Co., St. Louis, MO, USA) at 37°C in a humidified incubator with a 5% CO_2/95%
91 air atmosphere. Cells were sub-cultured every 3 days until a maximum passage of 40 was
92 reached.

93 *2.2. Cell Viability Assay*

94 Cell viability was tested by the MTT assay. Cells were seeded in a 96-well plate (2×10^4
95 cells/well) for 24 h, washed with PBS (Phosphate Buffered Saline; Welgene, Gyeongsan,
96 Korea), and treated with or without pectolinarin (30 μM) or pectolinarigenin (30 μM). After
97 72 h of incubation, the MTT reagent was added to each well, and the plate was incubated at
98 37°C for 2 h. The medium was discarded, and the plate was washed with PBS. The
99 intracellular formazan was dissolved in DMSO (Dimethyl sulfoxide; Sigma-Aldrich Co.) and
100 absorbance measured at 595 nm using a microplate reader (BIO-TEK Power Wave XS,
101 Winooski, VT, USA).

102 *2.3. Melanin Content Measurements*

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

109 *2.4. Measurement of intracellular tyrosinase activity*

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

120 *2.5. Western blotting analysis*

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

133 4000 film (Fuji film, Tokyo, Japan). An anti-GAPDH antibody was used to monitor protein
134 loading in each lane. Densitometric analysis was performed using the ImageJ software.

135 *2.6. Quantitative real-time PCR (Q-PCR) analysis of mRNA expression*

136 Total RNA was extracted using an RNeasy mini kit (Qiagen, Valencia, CA, USA) according to
137 the manufacturer's protocols and stored at -80°C until use. cDNA was amplified using a
138 ImProm-II Reverse Transcription System (Promega, Madison, WI, USA) according to the
139 manufacturer's guidelines. Then, SYBR green-based quantitative PCR was performed using
140 the Applied Biosystems 7500 Fast Real-Time PCR system and Fast SYBR® Green Master Mix
141 (Life technologies, UK). All reactions were run in triplicate, and the data were analyzed using
142 the $2^{-\Delta\Delta C_T}$ values method.[29] The sequences of the primers used in this study were MITF
143 forward: 5'-ATG GAC GAC ACC CTT TCT C-3'; MITF reverse: 5'-GGA GGA TTC GCT AAC AAG TG-
144 3'; Tyrosinase forward: 5'-GGC CAG CTT TCA GGC AGA GGT-3'; Tyrosinase reverse: 5'-TGG TGC
145 TTC ATG GGC AAA ATC-3'; TRP1 forward: 5'-AAG CAG ACA TCC AAC AAC ACT AG-3'; TRP1
146 reverse: 5'-GCA AGA GTT CAG AAC ACA GGT C-3'; TRP2 forward: 5'-GCA AGA GAT ACA CGG
147 AGG AAG-3'; TRP2 reverse: 5'-CTA AGG CAT CAT CAT CAT CAC TAC-3'; β-ctin forward: 5'-GAC
148 AGG ATG CAG AAG GAG ATT ACT-3'; β-actin reverse: 5'- TGA TCC ACA TCT GCT GGA AGG T-3'.
149 The quantity of each transcript was calculated as described in the instrument manual and
150 normalized to the amount of β-actin.

151 *2.7. Evaluation of inhibitory efficacy of PG on the reconstructed skin model*

152 A reconstructed human skin model (Neoderm®-ME; Tego Science, Seoul, Korea) consisting
153 of human epidermal melanocytes and human-derived epidermal keratinocytes was
154 incubated in serum-free maintenance medium (Tego Science). The reconstructed human skin
155 was incubated in the presence of pectolinarigenin (30 μM) for 2 days. Reconstructed human

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156 epidermis was fixed in 10% formalin, blocked in paraffin and stained for melanin using L-
157 DOPA and the Fontana-Masson staining method.

158 *2.8. Plant material and extraction*

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

166 *2.9. Hydrolysis of hot-water C. setidens extract*

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

173 *2.10. LC/MS Analyses*

174 Test samples were analyzed for pectolinarin and pectolinarigenin using the LC/MS method
175 reported previously.[19] The LC/MS system consisted of an Agilent 1200 Series HPLC system
176 (Agilent Technologies, Palo Alto, CA, USA), equipped with ESI mode. Test samples and
177 standard compounds were dissolved in MeOH using an ultrasonicator and a vortex mixer and
178 were then filtered through a 0.45 µm syringe filter before injection. The mobile phase was a

179 mixed solvent of 0.05% trifluoroacetic acid (TFA) in acetonitrile (solvent A) and 0.05% TFA in
180 water (solvent B). The gradient elution system was as follows: (A)/(B) = 10/90 (0 min; hold for
181 3 min) → 90/10 (13 min) →90/10 (13 min; hold for 5 min) → 95/5 (20 min). Column
182 temperature was maintained at 40°C using a temperature controller. Analysis was performed
183 at a flow rate of 1.00 mL/min, with the detection wavelength fixed at 340 nm.

184 *2.11. Statistical Analysis*

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

188

189 **3. Results**

190 *3.1. Inhibitory effects of Pectolinarin and pectolinarigenin on melanin biosynthesis in melan-a*
191 *cells*

192 To study the effects of pectolinarin and pectolinarigenin (Fig. 1A) on melanin biosynthesis,
193 after treatment with pectolinarin and pectolinarigenin at 30 μM for 72 h, the melanin
194 content and viability were measured. Neither pectolinarin nor pectolinarigenin showed
195 cytotoxicity at indicated concentrations. Treating melan-a cells with pectolinarin (30 μM) or
196 pectolinarigenin (30 μM) reduced the melanin content to 17.4 ± 6.2% and 31.5 ± 2.2%,
197 respectively, compared with controls (Fig. 1B and C). These results showed that
198 pectolinarigenin suppressed melanin biosynthesis without cytotoxicity to a significantly
199 higher degree than did pectolinarin treatment of melan-a cells. Reduced tyrosinase activity
200 in melan-a cells could be produced either by the repression of tyrosinase gene expression or
201 by the direct inhibition of tyrosinase. Cell lysates treated with pectolinarin and

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202 pectolinarigenin were utilized as tyrosinase sources. Intracellular tyrosinase activity was
203 suppressed by pectolinarigenin treatment, but pectolinarin did not inhibit intracellular
204 tyrosinase activity in melan-a cells. Treating melan-a cells with pectolinarigenin (30 μM)
205 decreased the intracellular tyrosinase activity to 46.9 ± 4.5% of the control group (Fig. 1D).

206 *3.2. The effects of pectolinarin and pectolinarigenin on melanin biosynthesis-related proteins*
207 *and gene expression in melan-a cells*

208 We investigated the effect that pectolinarin and pectolinarigenin had on expression of
209 melanin biosynthesis-related proteins, MITF, tyrosinase, TRP1, and TRP2, by western blotting
210 using cell lysates treated with pectolinarin and pectolinarigenin. Pectolinarigenin reduced the
211 protein expression of Tyrosinase, TRP1, and TRP2 by 37.9 ± 3.2%, 42.0 ± 2.6%, and 38.3 ±
212 3.8%, respectively, compared with the control group (Fig. 2A and B). MITF is the most
213 important transcription factor that regulates the expression of melanin biosynthesis-related
214 genes.[30] Pectolinarigenin dramatically suppressed MITF protein expression by 51.5 ± 8.5%
215 in melan-a cells. It has been suggested that the down-regulation of melanin biosynthesis-
216 related proteins by pectolinarigenin might be related to MITF signaling. To investigate
217 whether the inhibition of protein expression was associated with reduced the mRNA levels of
218 melanin biosynthesis-related genes, the mRNA expression levels of Tyrosinase, TRP-1, TRP-2,
219 and MITF were confirmed using Q-PCR. Pectolinarigenin reduced the gene expression of
220 Tyrosinase, TRP-1 and MITF by 55.0 ± 6.1%, 10.7 ± 3.3%, and 32.3 ± 1.0%, respectively (Fig.
221 2C). These results indicate that the presence of pectolinarigenin suppressed the expression
222 of tyrosinase-related genes, suggesting a mechanism of down-regulated MITF transcription.

223 *3.3. Effect of melanin biosynthesis by pectolinarigenin in a reconstructed human skin model*

224 To approximate human usage, a reconstructed human skin model was used to identify the

225 depigmenting effect of pectolinarigenin. The melanin content was significantly reduced in
226 pectolinarigenin-treated reconstructed human skin compared with the control group
227 according to visual and spectrophotometric evaluations (Fig. 3A). Treating reconstructed
228 human skin with pectolinarigenin decreased the melanin content to 20.8% of the control
229 group (Fig. 3B). Additionally, treating reconstructed human skin with pectolinarigenin
230 decreased the L-DOPA content compared with the control group (Fig. 3C). These results
231 imply pectolinarigenin is an anti-melanogenic material and has useful applications in human
232 skin.

233 *3.4. Conversion of Pectolinarin to Pectolinarigenin.*

234 For the simultaneous determination of pectolinarin and pectolinarigenin in *C. setidens* hot-
235 water extract, the optimized chromatographic conditions were investigated. Peaks of 2
236 compounds in the chromatograms were determined by comparing on-line UV spectra and
237 the retention times with those of the standards. Retention time for pectolinarin and
238 pectolinarigenin of *C. setidens* hot-water extract were 10.33 and 13.57 min, respectively (Fig.
239 4A). *C. setidens* hot-water extract with microwave hydrolysis (205°C) increased
240 pectolinarigenin contents 66.3% compared with that of the control group. In comparison,
241 pectolinarin content was decreased to 14.5% (Fig. 4B). These results indicate that microwave
242 irradiation efficiently converted the pectolinarin into pectolinarigenin. Additionally, this
243 reaction occurred more efficiently under the 1% acetic acid conditions than in neutral
244 solution. These results suggest that microwave irradiation under 1% acetic acid conditions is
245 optimal to convert pectolinarin of *C. setidens* hot-water extract into pectolinarigenin.

246

247 **4. Discussion**

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

259 We performed *in vitro* tests to investigate anti-melanogenic effects by pectolinarin and
260 pectolinarigenin. Both pectolinarin and pectolinarigenin inhibited melanin synthesis without
261 cytotoxicity in melan-a cells. Pectolinarigenin treatment showed more potent inhibitory
262 activity of melanin synthesis than did pectolinarin treatment. In agreement with the
263 observed effects on melanin content, the intracellular tyrosinase activity was also
264 significantly reduced by pectolinarigenin. However, treatment with pectolinarin increased
265 the intracellular tyrosinase activity. As a result, pectolinarigenin was effective at inhibiting
266 the signs related to melanogenesis in melan-a cells.

267 We studied the effects that pectolinarin and pectolinarigenin had on the expression of
268 melanin biosynthesis-related proteins MITF, Tyrosinase, TRP-1, and TRP-2, by western
269 blotting. Only pectolinarigenin inhibited the protein expression of MITF, tyrosinase, TRP-1,
270 and TRP-2. MITF is a key transcription factor that regulates the expression of most

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271 melanogenesis-related genes.[30] Pectolinarigenin dramatically suppressed MITF mRNA and
272 protein expression in melan-a cells. This finding suggests that pectolinarigenin could be
273 associated with altered MITF signaling to down-regulate melanogenesis-related proteins.
274 However, treatment with pectolinarin did not suppress melanogenesis-related proteins and
275 genes. There are significant differences between the anti-melanogenic effects of the two
276 compounds.

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

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

294 we identified that microwave irradiation under 1% acetic acid was the optimum sugar
295 elimination method. These results indicate that pectolinarin could be isolated from gondre
296 byproducts as a primary compound used for anti-melanogenesis activity and then converted
297 into pectolinarigenin via microwave irradiation.

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

307

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311

312 Conflict of interest

313 The authors declare no competing financial interests.

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404 **Figure legends**

405

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

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

419 **FIG. 3.** The inhibitory effect of pectolinarigenin on melanin production in a reconstructed
420 human skin model. (A) Histopathological features of Pigmentation and L-DOPA in
421 reconstructed skin. The sections were stained with Fontana-Masson (FM) and L-DOPA
422 (magnification, 100×). Melanin (B) and L-DOPA (C) contents were determined using ImageJ
423 software. The results are expressed as the mean ± S.D. of three randomly selected sites. *P <
424 0.05 vs. control (CON). AA: Ascorbic acid, PG: pectolinarigenin.

425 **FIG. 4.** The conversion effect of pectolinarin to pectolinarigenin by microwave irradiation. (A)
426 HPLC chromatogram of *C. setidens* hot-water extract and microwave irradiated extract. The

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427 presence of PN (t_R 10.33 min) and PG (t_R 13.57 min), in extracts were verified by comparing
428 each retention time and UV spectrum. (B) The efficiency of conversion to PG by microwave
429 irradiation and under acidic conditions.

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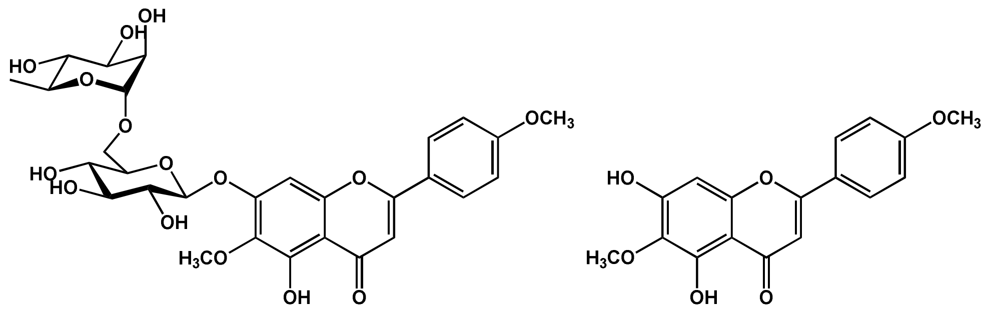
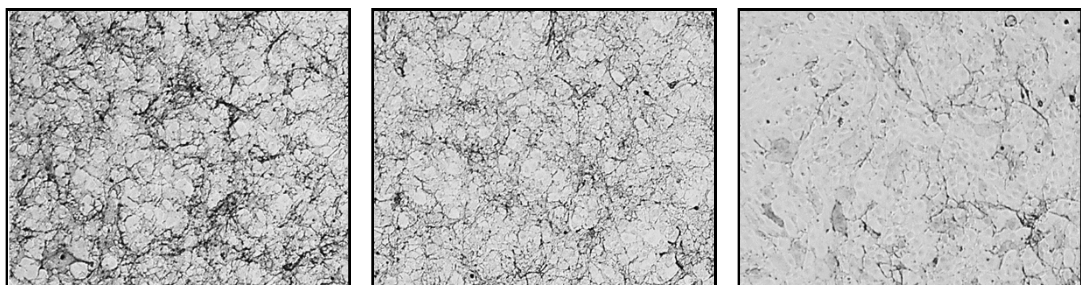
FIG. 1.

(A)



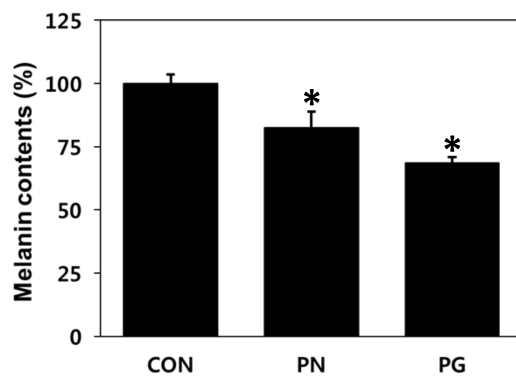
Pectolinarin **Pectolinarigenin**

(B)



CON **Pectolinarin** **Pectolinarigenin**

(C)



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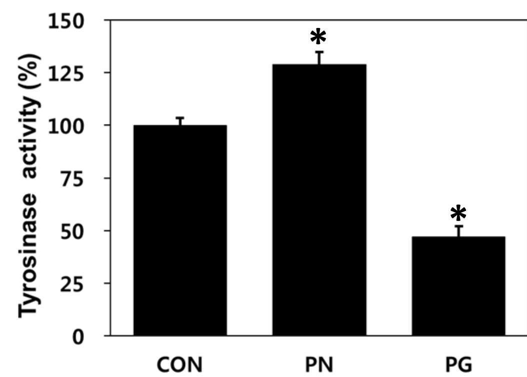
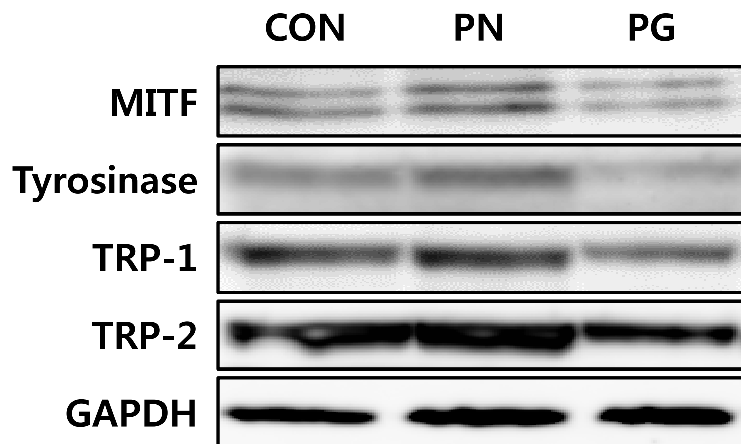


FIG. 2.

(A)



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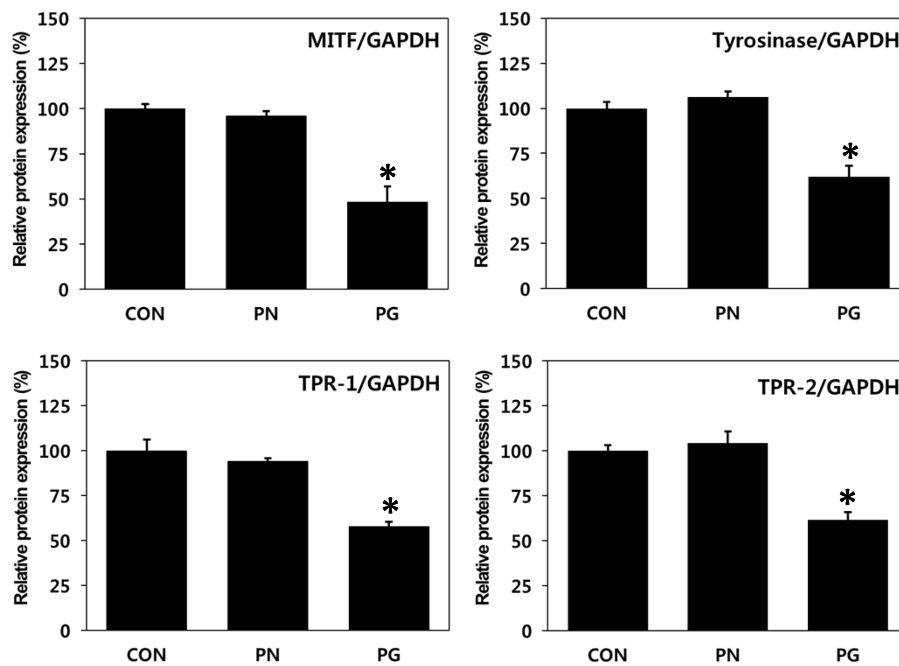

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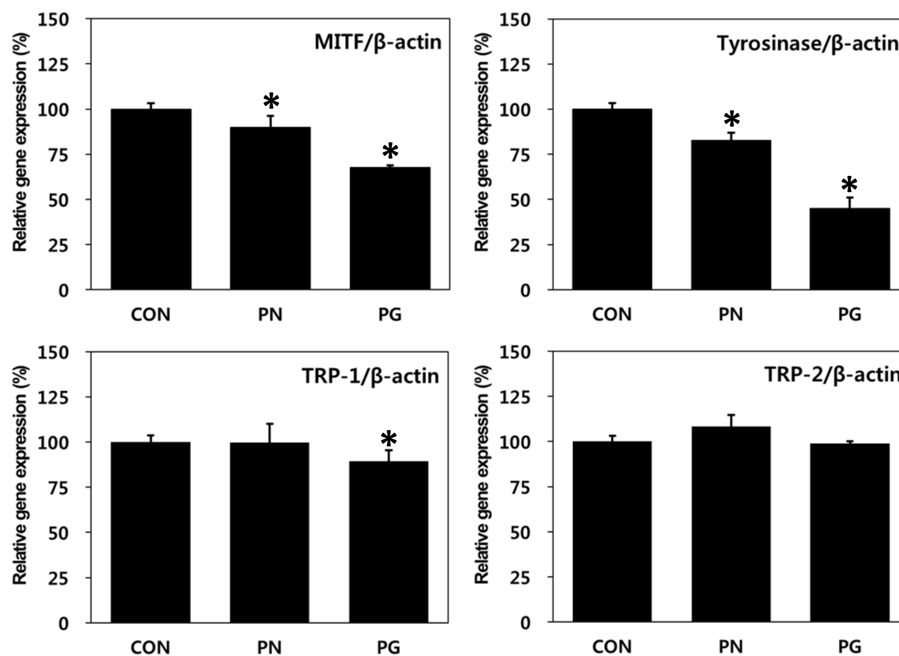
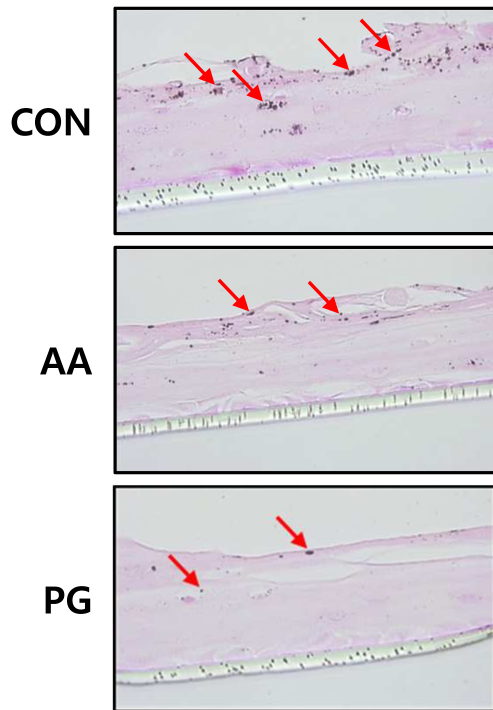
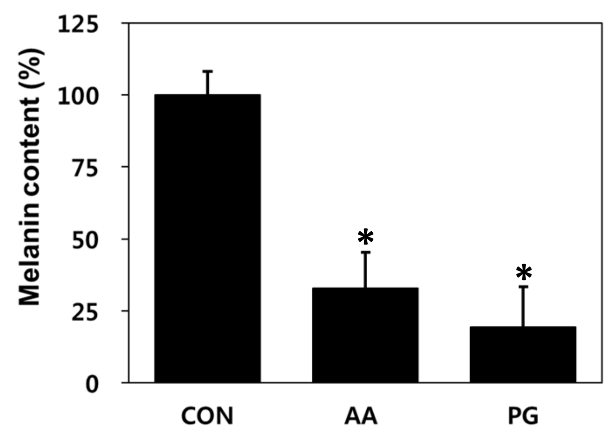
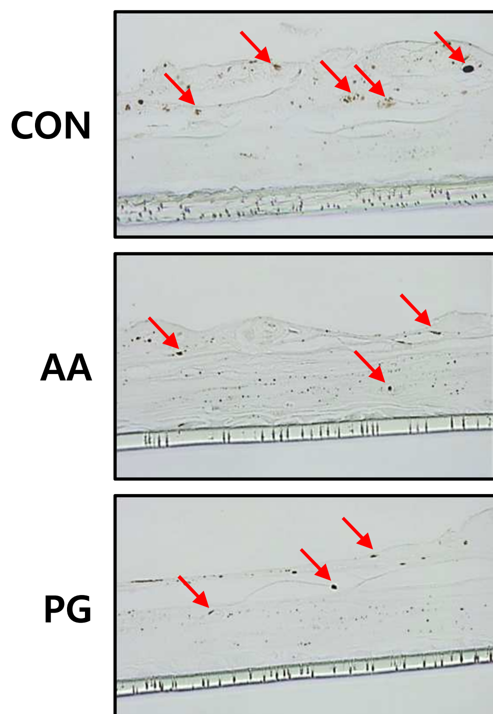


FIG. 3.







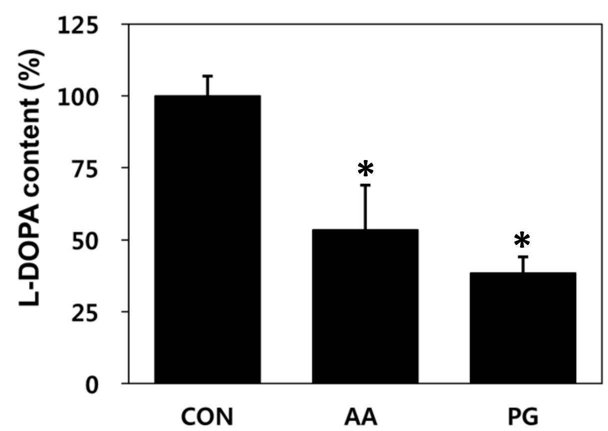
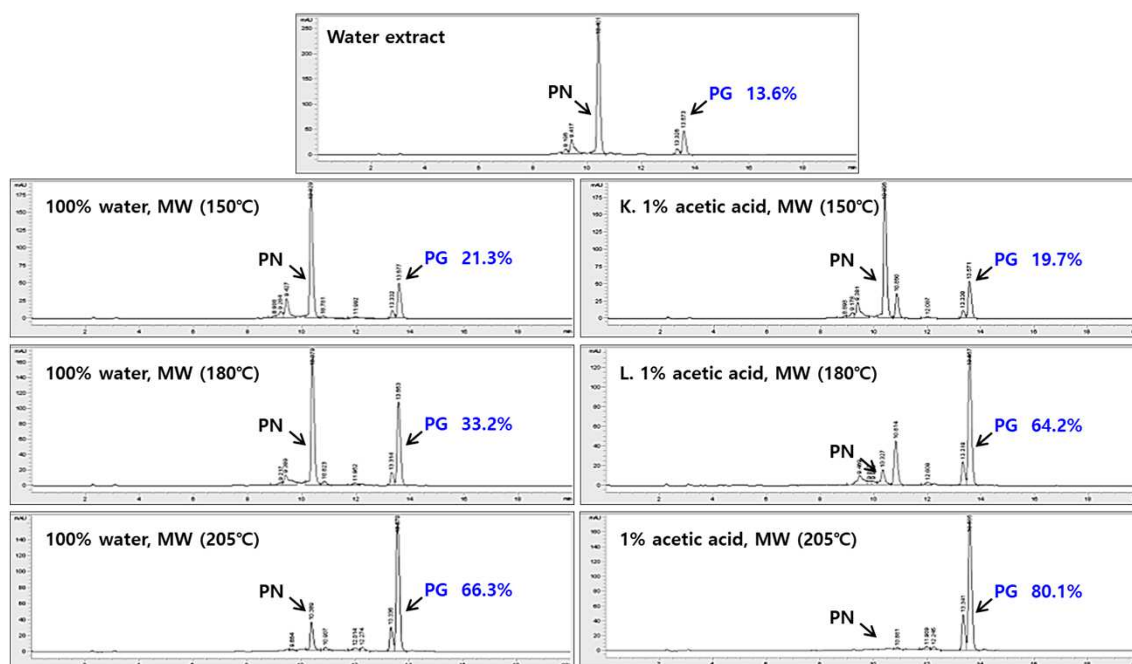
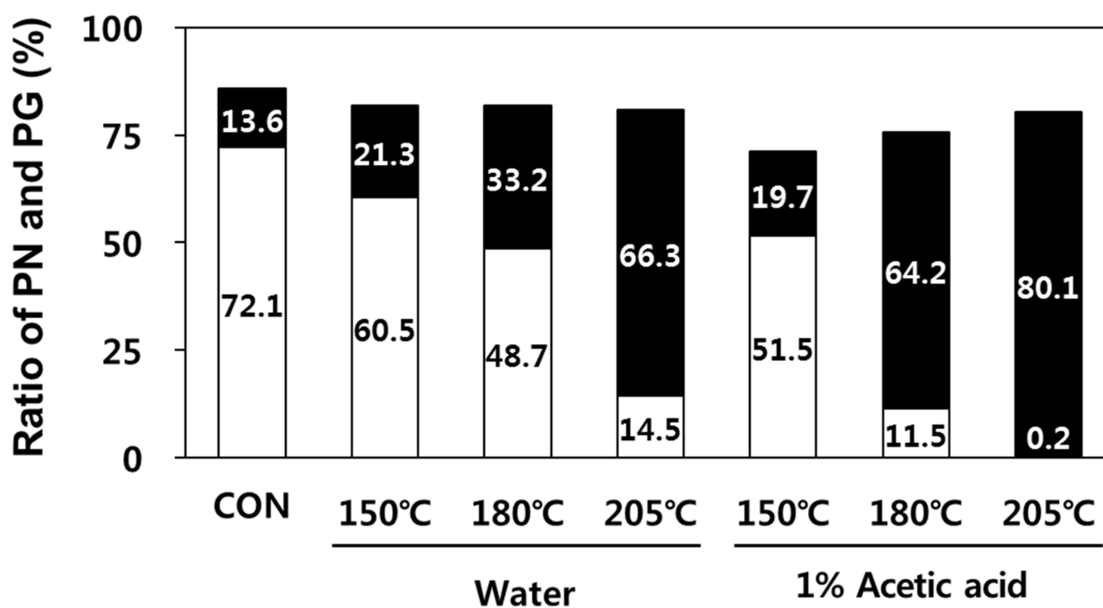


FIG. 4.

(A)



(B)



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Highlights

1. Pectolinarigenin has more potent inhibitory effects than pectolinarin on melanin synthesis in melan-a cells.
2. Pectolinarigenin has more potent inhibitory effects than pectolinarin on expression of melanogenesis-related protein and mRNA.
3. Pectolinarigenin has inhibitory effect on melanin production in a reconstructed human skin model.
4. Microwave irradiation under 1% acetic acid conditions at 205℃ and 100 watts is optimal to convert pectolinarin of *C. setidens* hot-water extract into pectolinarigenin.

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