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Melanin Synthesis Inhibition and Radical Scavenging Activities of Compounds Isolated from the Aerial Part of *Lespedeza cyrtobotrya*

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The EtOAc fraction of *Lespedeza cyrtobotrya* showed mushroom tyrosinase inhibitory and radical scavenging activities. Four active compounds were isolated based on Sephadex LH-20 chromatography and HPLC, and the structures were elucidated, on the basis of their LC–MS and NMR spectral data, as 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran (1), eriodictyol-7-*O*-glucopyranoside (2), haginin A (3), and dalbergioidin (4), respectively. Compound (1) showed mushroom tyrosinase inhibitory activity with an IC_{50} value of 5.2 μM and acted as a competitive inhibitor. Furthermore, 37.3 μM of compound 1 reduced 50% of the melanin content on human melanoma (MNT-1) cells. The radical scavenging activities of compounds 1, 2, 3, and 4 were shown to have IC_{50} values of 11.0, 24.5, 9.0, and 36.5 μM, respectively, in an ABTS system and IC_{50} values of 42.7, 36.0, 37.7, and 61.7 μM, respectively, in a DPPH system. The mushroom tyrosinase inhibitory activity of the EtOAc fraction of *Lespedeza cyrtobotrya* was contributed by compounds 1, 3, and 4, and its radical scavenging activity was contributed by compounds 1–4.

Keywords: Tyrosinase, human melanoma (MNT-1) cell, radical scavenging, *Lespedeza cyrtobotrya*

Melanin is a heterogeneous polyphenol-like biopolymer with a complex structure and color varying yellow to black [22]. In mammalian melanocytes, melanin is synthesized in melanosomes and plays an important role in protecting skin from the harmful effects of solar UV radiation. However, the accumulation of an abnormal melanin amount in the skin can lead to esthetic problems [24] such as the formation of chloasma or lentigo [17] as well as skin aging the onset of cutaneous cancer [1].

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Melanin is synthesized through the convertion of tyrosine, and tyrosinase plays a critical role in this process. Tyrosinase is known to be a key enzyme in melanin biosynthesis. The hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA), the oxidation of DOPA to dopaquinone, and the oxidation of 5,6-dihydroxyindole result in the formation of the indole 5,6-quinone. This enzyme has three domains, in which the central domain contains two copper binding sites and catalyzes three different reactions in the biosynthetic pathway of melanin in melanocytes [20]. During melanin biosynthesis, the reactive intermediate is oxidized to form melanin *via* a radical-coupling pathway. The melanocytes are under continuous low-grade oxidative stress [8]. Melanin synthesis results in the generation of hydrogen peroxide that, if inappropriately processed, can lead to the penetration of hydroxyl radicals and other reactive oxygen species (ROS). In the skin, tyrosinase can be regarded as an important system for the elimination of ROS [21]. That is, this enzyme is able to utilize ROS in the process of melanogenesis [25].

The inhibition of melanogenesis is a regulator of copper, an essential element of tyrosinase or tyrosinase-related protein (TRPs) [24]. Several of the well-known natural skin lightening and depigmentation agents, including kojic acid [11], arbutin [12], hydroquinone [4, 19], and azelaic acid [4], have been investigated for their inhibition mechanism and are currently being used as cosmetics addictives. However, some of these compounds are associated with side effects such as cytotoxicity and mutagenic effects [3].

Free-radical species and ROS can cause oxidative damage. Photooxidative damage to the skin occurs regularly owing to skin contact with oxygen and exposure to UV radiation [17]. The damage caused by excessive ROS and free radicals is casually linked to skin disorders [27] and is known to contribute to skin aging. Thus, antioxidants such as radical scavengers may have a beneficial effect on skin health [8].

In the continuing search for new effective melanogenesis inhibitors as skin whitening agents from natural sources, the focus was on the *Lespedeza* species. They are distributed across East Asia as well as North America, with the compounds typically used as folk medicines. In particular, it is used as a vermicide and for the treatment of dermatosis. Some bioactive compounds isolated from the ethyl-acetate-soluble fraction of a methanolic extract of the branch of *Lespedeza cyrtobotrya* showed tyrosinase inhibitory activity [2]. However, investigations of the inhibitory effect of some of these compounds on melanogenesis and of the antioxidant activity of the other compounds have not been completed.

Thus, in the current work, a number of compounds isolated from *Lespedeza cyrtobotrya* were screened using an *in vitro* mushroom tyrosinase and human melanoma (MNT-1) cells assay for radical scavenging activity.

Materials and Methods

General Instrumentation

All NMR experiments were recorded at 298 K on a Varian VnmrS 600 spectrometer (Varian Inc., U.S.A.) at the Korea Basic Science Institute (KBSI). 1D NMR spectra (^{1}H: 600 MHz; ^{13}C: 150 MHz; in methanol-d_4) were obtained with TMS as an internal standard. Chemical shifts (δ) and coupling constants (*J*) are all expressed in ppm and Hz, respectively. LC–ESI–IT–MS (electrospray ionization ion-trap mass spectrometry) data were collected on a Varian 500-MS ion-trap spectrometer (Varian Inc., U.S.A.). Preparative HPLC was performed using an instrument manufactured by Hitachi (Hitachi Inc., Japan). Column chromatography was accomplished *via* a Sephadex LH-20 device (GE Healthcare Bio-Sciences AB).

Chemicals and Reagents

Mushroom tyrosinase (E.C. 1.14.18.1), L-tyrosine, 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and crystal violet were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetonitrile and water were purchased from Burdick & Jackson (Muskegon, MI, U.S.A.). Methanol-d_4 was purchased from Cambridge Isotope Laboratories Inc. (Miami, FL, U.S.A.). Dulbecco's Modified Eagle's Medium (DMEM), trypsin-EDTA, and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, U.S.A.). Phosphate-buffered saline (PBS) was purchased from Mediatech, Inc. (Herndon, VA, U.S.A.).

Plant Material, Extraction, Isolation, and Structure Determination

The methanol extract from the aerial parts of *Lespedeza cyrtobotrya* was obtained from the Plant Extract Bank at KRIBB (Daejeon, Korea).

The methanol extract of *L. cyrtobotrya* (3 g) was diluted with water (400 ml) and then partitioned with ethyl acetate (EtOAc) and butanol (BuOH). Among the solvent fractions, the EtOAc fraction showed many peaks on a chromatogram obtained *via* high-performance liquid chromatography analysis and which had the most inhibitory effects on mushroom tyrosinase activity than the other fractions. Accordingly, the EtOAc fraction was concentrated under a vacuum. The residue was fractionated *via* Sephadex LH-20 column chromatography using aqueous 80% MeOH as a solvent. To obtain the pure bioactive compounds **1–4**, final purification was accomplished *via* preparative HPLC using C_{18} reversed-phase column (YMC-Pack Pro C18 250× 10 mm I.D., 5 μm). The mobile phase consisted of 5% acetonitrile in water (A) and acetonitrile (B) using a gradient program of 95–0% A and 0–95% B in 60 min. The flow rate was 3.0 ml/min and absorbance was detected at 220 nm.

2-(2,4-Dihydroxyphenyl)-6-hydroxybenzofuran (compound **1**): Dark-brown powder; UV λ_{max} (MeOH): 218, 282, 320 nm. ^{1}H-NMR (600 MHz, Methanol-d_4) δ: 6.9 (1H, s, H-3), 7.6 (1H, d, *J*=8.4 Hz, H-4), 6.7 (1H, dd, *J*=8.4, 2.4 Hz, H-5), 6.9 (1H, d, *J*=2.4 Hz, H-7), 7.0 (1H, d, *J*=2.4 Hz, H-3'), 6.4 (1H, dd, *J*=8.4, 2.4 Hz, H-5'), 7.3 (1H, d, *J*=8.4 Hz, H-6'); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 157.1 (C-2), 104.0 (C-3), 124.1 (C-4), 112.7 (C-5), 153.8 (C-6), 98.4 (C-7), 156.1 (C-8), 121.6 (C-9), 108.2 (C-1'), 156.1 (C-2'), 104.1 (C-3'), 159.6 (C-4'), 111.7 (C-5'), 128.2 (C-6'); Major long range ^{1}H–^{13}C correlations (HMBC) of observed as H-2/C-1', C-2', C-6'; H-3/C-2, C-4, C-1'; H-6/C-5, C-7, C-8; H-1''/C-7, C-2'', C-3''; ESI–MS: *m/z*: 241 $[M-H]^-$. The molecular formula and weight were determined by the ^{1}H-NMR, ^{13}C-NMR, and ESI–MS spectral data. Accordingly, the purified compound was identified as 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran (molecular formula $C_{14}H_{10}O_4$; molecular weight 242) (Fig. 1A) [14].

Eriodictyol-7-*O*-glucopyranoside (compound **2**): Yellow powder; UV λ_{max} (MeOH): 226, 282 nm. ^{1}H-NMR: (600 MHz, Methanol-d_4) δ: 5.3 (1H, d, *J*=13.2 Hz, H-2), 3.1 (2H, dd, *J*=12.6, 13.2 Hz, H-3), 6.2 (1H, s, H-6), 6.2 (1H, s, H-8), 6.9 (1H, s, H-2'), 6.8 (1H, s, H-

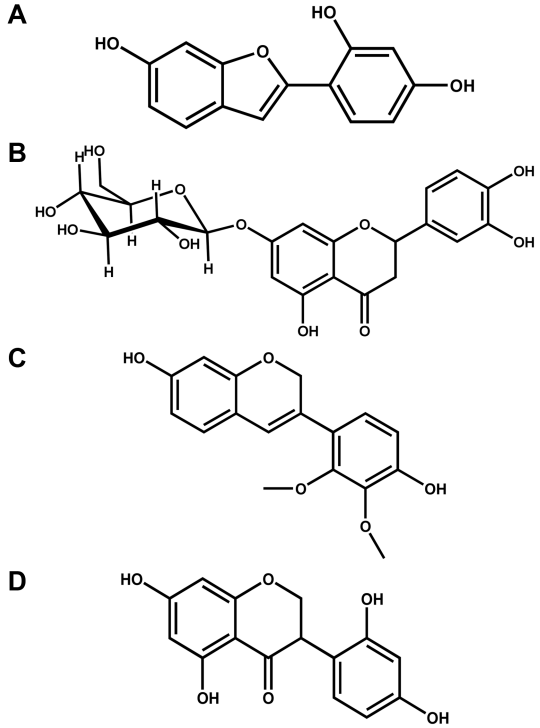

Fig. 1. The structures of compounds **1–4** (**A–D**, respectively).
Compound **1**: 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran; Compound **2**: eriodictyol-7-*O*-glucopyranoside; Compound **3**: haginin A; Compound **4**: dalbergioidin.

5'), 6.8 (1H, s, H-6'), 5.0 (2H, t, H-1"), 3.4-3.5 (3H, overlapped, H-2", 3", 5"), 3.4 (1H, t, H-4"), 3.7 (1H, m, H-6"), 3.9 (1H, d, *J*=10.2, H-6"); ^{13}C-NMR (150 MHz, Methanol-d_4) δ: 80.7, 80.8 (C-2), 44.2, 44.4 (C-3), 198.7 (C-4), 165.0, 165.1 (C-5), 97.1 (C-6), 167.1, 167.2 (C-7), 98.1 (C-8), 164.7 (C-9), 105.0 (C-10), 131.6, 131.7 (C-1'), 114.9 (C-2'), 147.1 (C-3'), 146.7 (C-4'), 116.4 (C-5'), 119.5 (C-6'), 101.3, 101.4 (C-1"), 74.8 (C-2"), 78.4 (C-3"), 71.3 (C-4"), 77.9 (C-5"), 62.5 (C-6"); ESI−MS: *m/z*: 449 $[M-H]^-$. The molecular formula and weight were determined by the ^{1}H-NMR, ^{13}C-NMR, and ESI−MS spectral data. The six twin carbons of the ^{13}C-NMR spectrum indicated it to be two stereoisomers. The HMBC correlations of the anomeric protons to the C-7 twin carbons (δ 167.1, 167.2) confirmed the linkage of the sugar residue to the C-7 of flavanone aglycones. Accordingly, the purified compound was identified as eriodictyol-7-*O*-glucopyranoside (molecular formula $C_{21}H_{22}O_{11}$; molecular weight 450) (Fig. 1B) [16].

Haginin A (compound **3**): colorless needles; UV λ_{max} (MeOH): 243, 322 nm. ESI−MS: *m/z*: 299 $[M-H]^-$. The purified compound was identified as haginin A (molecular formula $C_{17}H_{16}O_5$; molecular weight 300) by comparison with previously collected data (Fig. 1C) [13].

Dalbergioidin (compound **4**): Dark-brown powder; UV λ_{max} (MeOH): 230, 290 nm. ESI−MS: *m/z*: 287 $[M-H]^-$. The purified compound was identified as dalbergioidin (molecular formula $C_{15}H_{12}O_6$; molecular weight 288) by comparison with previously collected data (Fig. 1D) [7].

Tyrosinase Inhibitory Activity

Tyrosinase activity in MNT-1 cells was determined by a modified method [18]. MNT-1 cells were cultured in a 6-well plate. They were then replaced for 4 days with a fresh medium containing various concentrations of compounds. Then, the cells were washed in ice-cold PBS and lysed with phosphate buffer (pH 6.8) containing 1% Triton X-100. The lysates were clarified by centrifugation for 5 min at 10,000 ×*g*. After the quantification of protein levels and the regulation of concentrations using lysis buffer, 90 μl of each lysate, which contained identical amounts of protein, and 10 μl of 15 mM L-DOPA were added to a 96-well microplate. After incubation at 37°C, absorbance was measured every 10 min for at least 1 h, at 490 nm, with a microplate reader. A cell-free assay system was used to test for the direct effects of compound **1** on mushroom tyrosinase. The mushroom tyrosinase inhibitory activity was determined as described previously with some modification [7]. The reaction mixture consisted of 151 μl of 0.1 M sodium phosphate buffer (pH 6.5), 5 μl of dissolved sample in DMSO, 8 μl of mushroom tyrosinase (2,500 unit/ml), and 36 μl of 1.5 mM L-tyrosine added to a 96-well microplate. After the reaction mixture was incubated at 37°C for 20 min, the absorbance was measured at 490 nm with a microplate reader. The activity was expressed as the half maximal inhibitory concentration of the enzyme activity (IC_{50}).

Kinetic Analysis of Tyrosinase Inhibition

Using various concentrations of L-tyrosine (1.7 to 5 mM) as a substrate, mushroom tyrosinase (2,500 unit/ml) and 0.1 M sodium phosphate buffer (pH 6.5) with test samples (0.434 and 0.868 μM) were added to a 96-well microplate in a total volume of 200 μl. The initial rate of dopachrome formation from the reaction mixture was determined by the absorbance level at a wavelength of 490 nm per minute using a microplate reader. The Michaelis constant (K_m) and maximal velocity (V_{max}) kinetic parameters of the tyrosinase activity were determined using a Lineweaver−Burk plot [5]. The reaction kinetics required modification of the Michaelis−Menten equation owing to competitive inhibition by compound **1** together with substrate inhibition by L-tyrosine [6].

Cell Culture

Pigmented human melanoma (MNT-1) cells were kindly provided by Vincent Hearing, NIH and were cultured in DMEM with a 10% fetal bovine serum (FBS), mixture of streptomycin/penicillin (100 μg/ml each) at 37°C in 5% CO_2. Cells were subcultured every 3 days. The cells were harvested with a mixture of 0.05% trypsin and 0.53 mM EDTA.

Cell Viability

Cell viability was determined using crystal violet. The cells were seeded at a density of 1×10^5 cells per well in 96-well plates. They were then for 4 days replaced with a fresh medium containing various concentrations of compounds. Then, the cultured medium was removed and replaced with 0.1% crystal violet in 10% ethanol. The cells were stained for 5 min at room temperature and rinsed three times. The crystal violet retained by the adherent cells was then extracted using 95% ethanol. The plate was measured at 570 nm.

Inhibition of Melanogenesis in MNT-1 Melanoma Cells

The cells were seeded in a 24-well plate at a density of 1×10^5 cells per well and allowed to attach overnight. They were then incubated for 4 days at various concentrations. After being washed with PBS, the cells were lysed with 250 μl of 0.85 N KOH and transferred to a 96-well plate. The melanin contents were estimated by measuring the absorbance level at 405 nm [10].

DPPH Radical Scavenging Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was adopted with modifications [28]. The reaction mixture consisted of 180 μl of an ethanolic solution of 200 μM DPPH and a 20 μl sample solution in a 96-well microplate. It was incubated at 37°C for 20 min. After incubation, the absorbance level was measured at 515 nm using a microplate reader.

ABTS Radical Cation Decolorization Assay

The 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation decolorization assay employed here was similar to that described by Re *et al*. [23]. The ABTS radical cation ($ABTS^{\cdot+}$) was prepared by incubation of a potassium persulfate (2.45 mM)-containing ABTS stock solution (7 mM) in the dark at room temperature. The $ABTS^{\cdot+}$ solution was diluted with water until an absorbance of 0.7 at 750 nm was reached. Then, 180 μl of the $ABTS^{\cdot+}$ solution was mixed with a 20-μl sample solution in a 96-well microplate and then incubated at 37°C for 6 min. After incubation, the absorbance level was measured at 750 nm using a microplate reader.

RESULTS

Mushroom Tyrosinase Inhibitory Activity of Compound 1

The mushroom tyrosinase inhibitory effect of compound **1** was increased as its concentration levels increased. This showed an inhibitory effect on mushroom tyrosinase activity with an IC_{50} value of 5.2 μM. Compound **1** showed a higher potential inhibitory activity compared with the

Table 1. Effects of compounds **1**–**4** on melanogenesis and radical scavenging.

	Tyrosinase inhibitory activity	Inhibition type	ABTS radical scavenging activity	DPPH radical scavenging activity	Melanin synthesis	Cytotoxicity	Reference
Compound	IC_{50} (μM)[a]	-	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	LD_{50} (μM)[b]	
1	5.2±3.9	Competitive	11.0±5.6	42.7±1.1	37.3±1.0[d]	149.4±2.8	-
2	1321.7±4.1	NT[c]	24.5±3.1	36.0±2.6	NT	NT	-
3	5.0±1.5	Noncompetitive	9.0±3.3	37.7±3.6	3.3[e]	14.5	[19]
4	20.0±3.5	Noncompetitive	36.5±1.8	61.7±1.5	27.7±2.1[e]	140±15.3	[15]
Kojic acid	50.1±19.6	NT	NT	NT	>200[e]	>200	-
Vitamin C	NT	NT	23.2±2.7	34.3±1.5	NT	NT	-

[a]IC_{50}; 50% inhibitory concentration. [b]LD_{50}; 50% lethal dose. [c]Not tested. [d]Tested with MNT-1 cell. [e]Tested with Melan-A cell. Kojic acid and Vitamin C were used as positive controls. Values are averages of triplicate experiments and expressed as the mean ± SD.

well-known tyrosinase inhibitor, kojic acid (IC_{50} 50.1 μM) (Table 1). Compound **1** was almost 10-fold more potent than kojic acid in its inhibitory effects on the *O*-hydroxylation of L-tyrosine.

Kinetic Parameters of Mushroom Tyrosinase in the Presence of Compound

A kinetic study of melanin synthesis by mushroom tyrosinase was conducted in the presence of compound **1** (Fig. 2). The Michaelis constant (K_m) and maximal velocity (V_{max}) of tyrosinase were determined using a Lineweaver–Burk plot with various concentrations of L-tyrosine as a substrate, as shown in earlier studied by the authors [2, 7]. With compound **1,** the V_{max} value (ΔA_{490}/min) was 0.2×10^{-1} and the K_m values increased in a dose-dependent manner (Table 2). The K_i values were estimated as 3.37×10^{-9} at 0.434 μM and 9.02×10^{-9} at 0.868 μM. The diagnostic criterion for competitive inhibition is the V_{max} value, which is constant as it is unaffected by the inhibitor. That is, all lines share a common y-intercept [5]. The results and reference established that the type of inhibition by compound **1** was competitive tyrosinase inhibition in the presence of L-tyrosine.

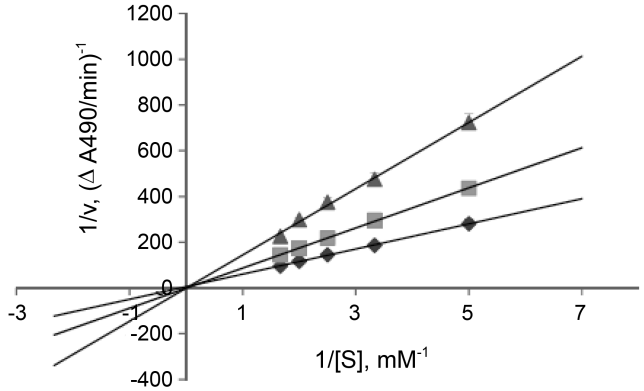

Fig. 2. Lineweaver–Burk plot of mushroom tyrosinase in the presence of compound **1**.
Data were obtained as mean values of $1/V$, inverse of the increase of absorbance at 490 nm per min (ΔA_{490}/min), of three independent tests with different concentrations of L-tyrosine as a substrate. Inhibitors of the enzyme were compound **1** 0.434 μM (square), 0.868 μM (triangle) and control (diamond). The modified Michaelis-Menten equation is $1/V_{max}=1/K_m(1+[I]/K_i)$. V for velocity of reaction, V_{max} for maximal velocity, S for L-tyrosine concentration, K_m for Michaelis constant, K_i for inhibitor constant.

Effects of Compound 1 on Melanogenesis and Cell Viability of Pigmented Human Melanoma (MNT-1) Cells

To investigate the inhibitory effect on melanin synthesis and the cytotoxicity on melanosome, 10–40 μM of compound **1** was exposed to MNT-1 cells for 4 days. Cell viability was then assessed using crystal violet assays. At doses of 10, 20, and 40 μM of compound **1**, the cell viabilities were 86.0%, 82.5%, and 75.9%, respectively (Fig. 3A). Kojic acid was used as a positive control in this study. Compound **1** showed LD_{50} at 149.4 μM on cell proliferation (Table 1). Melanin production in the cells was inhibited to 93.5% at 10 μM, 81.9% at 20 μM, and 42.2% at 40 μM (Fig. 3B). The IC_{50} value of melanin production in the cells was 37.3 μM with compound **1** (Table 1). Compound **1** has an effect on the melanin production of melanoma cells. In addition, compound **1** at the same concentrations also decreased tyrosinase activity in MNT-1 cells (Fig. 3C), and decreased pigmentation in the cells treated with compound **1** was observed (Fig. 3D).

Table 2. Kinetic parameters of mushroom tyrosinase in the presence of compound **1**.

Compound **1** (M)	K_m (M)	V_{max} (ΔA_{490}/min)	K_i (M)
None	1.06×10^{-2}	0.20×10^{-1}	-
4.34×10^{-7}	8.26×10^{-2}		3.37×10^{-9}
8.68×10^{-7}	11.2×10^{-2}		9.02×10^{-9}

The kinetic parameters were obtained, with L-tyrosine as a substrate, using the Lineweaver–Burk plot shown in Fig. 2. Values are averages of triplicate experiments and expressed as the mean ± SD.

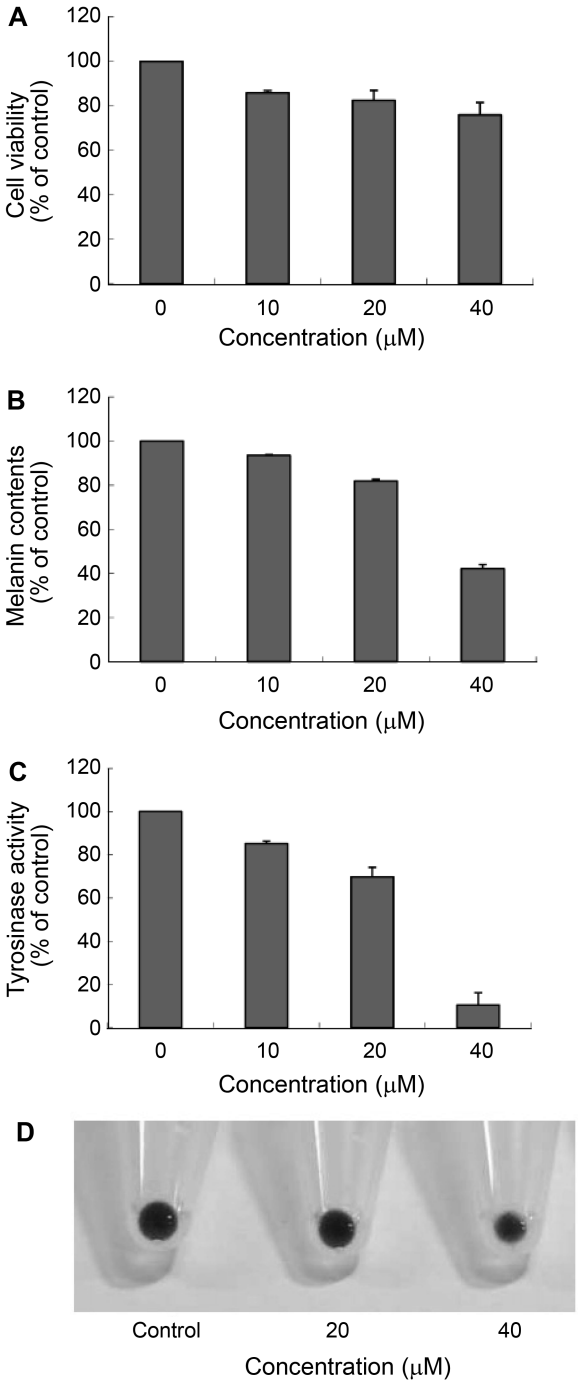

Fig. 3. Effects of compound **1** on melanogenesis in cultured MNT-1 cells.

The cells were cultured with 10–40 μM of compound **1** for 4 days. **A**. Cell viability; **B**. Melanin contents; **C**. Tyrosinase activity; and **D**. Photograph. Values are averages of triplicate experiments and expressed as the mean ± SD.

Radical Scavenging Activity of Compounds 1–4

The radical scavenging activity of compounds **1–4** was tested by measuring the ABTS and DPPH radical scavenging activities. The radical scavenging activity was presented as IC_{50} values in both the ABTS and DPPH systems. In the ABTS system, the radical scavenging activities (IC_{50}) of compounds **1–4** were found to be 11.0 μM, 24.5 μM, 9.0 μM, and 36.5 μM, respectively (Table 1). The activities of compounds **1** and **3** were similar and showed higher values compared with that of vitamin C. The radical scavenging activity of compound **3** was stronger than those of other compounds. In the DPPH system, the IC_{50} values of compounds **1–4** were 42.7 μM, 36.0 μM, 37.7 μM, and 61.7 μM, respectively. Compounds **2** and **3** showed similar radical scavenging activity in the DPPH system. Overall, the radical scavenging activities of compound **3** were stronger than those of other compounds. Compound **3** showed a more effective radical scavenging activity than the other bioactive compounds isolated from the aerial part of *Lespedeza cyrtobotrya*.

DISCUSSION

Some compounds of *Lespedeza cyrtobotrya* exhibited a good inhibitory effect on the mushroom tyrosinase compared with positive controls such as kojic acid or arbutin [2, 7, 15].

In previous research, the authors reported that compounds **3** and **4**, isolated from *Lespedeza cyrtobotrya*, have an inhibition activity on melanogenesis. However, the melanin biosynthesis inhibitory activity of compounds **1** and **2** and the radical scavenging activity of compounds **1–4** as *Lespedeza cyrtobotrya* metabolites have yet to be reported.

It was observed that the methanol extract of *Lespedeza cyrtobotrya* showed strong melanin biosynthesis inhibitory activity, and that the bioactive compound **1** (IC_{50} 5.2 μM) is 2-(2,4-dihydroxyphenyl)-6-hydroxybenzofuran. However, compound **2** has not shown inhibitory activity on melanogenesis. In the kinetics study, compound **1** was identified as a competitive inhibitor of the mushroom tyrosinase with the same V_{max} value. This was determined using compound **1** of 0.434 μM and 0.868 μM, where it was observed the inhibitory effect on mushroom tyrosinase using compound **1** of lower concentration than the IC_{50} value. This result was different to compounds **3** and **4** (Table 1). Therefore, it may bind at the same site as the copper active site of mushroom tyrosinase, in a case similar to that of quercetin and kaempferol [9, 26].

Moreover, in the human melanoma (MNT-1) cell system, the melanin contents and the tyrosinase activity were dramatically reduced in a dose-dependent manner by compound **1**. The inhibition concentration of melanogenesis was 37.3 μM at 50% inhibition by compound **1**, in comparison with an untreated control. This inhibitory effect of compound **1** was stronger than kojic acid. Moreover, the effective concentration of compound **1** did not lead to cytotoxicity within the activity concentrations. This result implies that as a potential skin whitening agent, it is relatively safe and does not influence cell growth (Table 1).

Using an antioxidant activity assay, four compounds of the aerial part of *Lespedeza cyrtobotrya* were found to have radical scavenging activity. 2-(2,4-Dihydroxyphenyl)-6-hydroxybenzofuran (**1**), eriodictyol-7-*O*-glucopyranoside (**2**), haginin A (**3**), and dalbergioidin (**4**) exhibited relatively high levels of radical scavenging activity. Among compounds **1**–**4**, compound **3** (IC_{50} 9.0 μM in the ABTS system and IC_{50} 37.7 μM in the DPPH system) and compound **1** (IC_{50} 11.0 μM in the ABTS system and IC_{50} 42.7 μM in the DPPH system) showed more effective radical scavenging activities than that of vitamin C as the positive control.

In conclusion, the findings of this study about the bioactive compounds obtained from the aerial part of *Lespedeza cyrtobotrya* denoted that compound **1** showed inhibitory activity on tyrosinase and melanogenesis in cells together with the previously reported compounds **3** and **4**. Compound **2** showed only radical scavenging activity; however, compounds **1**, **3**, and **4** showed both activities of tyrosinase inhibition and radical scavenging. Thus, it is proposed that the combination of these three melanin biosynthesis inhibitory and four radical scavenging compounds of *Lespedeza cyrtobotrya* extract could be a good candidate for a complex skin-whitening agent with antiaging effects.

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