ISSN (Online) 2287-3406
Journal of Life Science **2020** Vol. 30. No. 12. 1033~1041 DOI : https://doi.org/10.5352/JLS.2020.30.12.1033

The Effect of Inhibition of *Uncaria rhynchophylla* as an Inhibitor of Melanogenesis and an Antioxidant in B16F10 Melanoma Cells

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Received August 26, 2020 / Revised September 14, 2020 / Accepted September 15, 2020

Many people of all ages wish to have lighter skin for cosmetic reasons, and natural products attract more attention than chemically synthesized compounds. *Uncaria rhynchophylla* is widely used in Asia as a traditional herbal medicine. In order to find novel skin whitening agents, the present study evaluated the antioxidant activity and potential tyrosinase-inhibiting properties of *U. rhynchophylla*. Specifically, this study analyzed the antioxidant capacity of a 70% ethanolic extract of *U. rhynchophylla* as well as its effects on tyrosinase activity and melanin synthesis. Total mRNA levels were examined using reverse transcription polymerase chain reaction. The results revealed that *U. rhynchophylla* extracts exhibit great antioxidant capacity and significant levels of polyphenol and flavonoid compounds. *U. rhynchophylla* extracts can also powerfully inhibit tyrosinase activity. This same capacity was observed in melanoma B16F10 cells; that is, *U. rhynchophylla* extracts suppressed intracellular tyrosinase activity and reduced the amount of melanin in treated cells. In addition, a 1 mg/ml concentration of *U. rhynchophylla* extract significantly reduced the mRNA expression levels of tyrosinase. *U. rhynchophylla* extracts decrease tyrosinase and inhibit melanogenesis in B16F10 cells. This finding suggests that *U. rhynchophylla* has great potential as a natural whitening agent in skincare products.

Key words : Antioxidant, B16F10 melanoma cells, kojic acid, tyrosinase, *Uncaria rhynchophylla*

Introduction

Human skin color is mainly determined by amount of melanin, which is produced in the melanocytes, the pigment-producing cells of the epidermis. Melanin plays a crucial role in the response of the skin to the exposure of stress, ultraviolet light and melanin-stimulating factors like alpha-melanocyte-stimulating hormone (α-MSH) [21]. However, the overproduction and accumulation of melanin can lead to a variety of skin conditions, including freckles, chloasma, and other pigmentation syndromes [25, 29].

In melanin biosynthesis, the pathway of melanogenesis is known, and there are two types of melanin within the melanosomes: eumelanin and pheomelanin [4]. The first step of melanogenesis is initiated by tyrosine oxidation to dopaquinone catalyzed by the tyrosinase. Tyrosinase is the key enzyme in the first two steps, converting the hydroxylation of L-tyrosine to L-3, 4-dihydroxyphenylalanine (L-DOPA) and then to dopaquinone. Since tyrosinase is a rate-limiting enzyme that is produced only by melanocytic cells, a tyrosinase inhibitor can specifically target the melanin cells without other side effects [3]. This type of inhibitor has become highly popular as a whitening agent in cosmetics and pharmaceuticals. The hormone α-MSH is a peptide derived from proopiomelanocortin (POMC), which regulates melanogenesis via a cyclic adenosine monophosphate (c-AMP)-dependent pathway [2, 10]. When binding to its receptor, melanocortin receptor 1 (MC1R), up to a 100-fold increase in melanogenesis occurs [3].

Several skin-whitening chemicals used in cosmetics, such as kojic acid, arbutin, hydroquinone, aloesin, soybean extract, azelaic acid, licorice extract, niacinamide, and magnesium ascorbyl phosphate, have been reported to reduce melanogenesis [6, 8, 12, 17, 24, 27, 33]. Moreover, kojic acid is widely used in cosmetics and is often used as a positive

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control. The B16F10 melanoma cell line was considered a good model for investigating human melanoma as they are easy to culture in vitro, and they show most of the melanogenic mechanisms of melanocytes [3]. Hence, this cell line was chosen for this evaluation of natural melanin production inhibitors.

This study focuses on a new and potent natural source that shows great melanogenesis inhibitory activity. A member of the *Rubiaceae* family, *Uncaria* comprises numerous species worldwide, including in Southeast Asia, Central America, and South America. Uncaria rhynchophylla has been used in various prescriptions in traditional Chinese medicine (TCM) for the treatment of convulsive disorders (epilepsy) [9] and for various head ailments such as headaches and dizziness [13]. The dried stem and hook of *U. rhynchophylla* are named as Gou-Teng or cat's claw in TCM [16, 32]. Despite its common usage, there are few reports on the anti-pigmenting effect of *U. rhynchophylla*. Yon-Suk Kim3 et al. [15] demonstrated the antioxidant activity of and the presence of polyphenol and flavonoids in *U. rhynchophylla* extracts, and it has been proven that natural flavonoids are useful tyrosinase inhibitors [31]. Therefore, we hypothesized that *U. rhynchophylla* can potentially inhibit melanogenesis, though similar studies have not yet been reported on *U. rhynchophylla*. To test this hypothesis, assays were done on *U. rhynchophylla* to detect the inhibitory activity. Ethanolic extracts of Uncaria rhynchophylla were chosen for their melanogenesis inhibition on mushroom tyrosinase using L-DOPA as a substrate. The effect of *U. rhynchophylla* extracts on melanogenesis was evaluated by measuring melanin content and intracellular tyrosinase activity in melanoma B16F10 cells. Total mRNA levels were examined using reverse transcription polymerase chain reaction. In addition, the total polyphenols and flavonoids contents and antioxidant characteristics of the *U. rhynchophylla* extracts were analyzed.

Materials and Methods

Chemicals and reagents

Kojic acid, L-DOPA, tyrosine, 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT), diphenyl-1-picrylhydrazyl (DPPH), 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS)), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, U.S.).

Preparation of the *U. rhynchophylla* extracts

The stems and hooks of *U. rhynchophylla* were purchased from the Gukje Market of Busan, Korea in July 2019. The *U. rhynchophylla* (100 g) were through an extraction process with 1,000 ml 70% ethanol for 4 hr. The supernatant was collected by filtration and the extract was harvested by rotary evaporation. The resulting dried *U. rhynchophylla* extract powder was frozen at -80℃ for 7 days and stored at 4℃ for further use.

Antioxidant characteristics of the *U. rhynchophylla* extract

The antioxidant capacity of the *U. rhynchophylla* extract was determined by measuring the DPPH and ABTS scavenging activity as previously described [5, 22]. DPPH is a stable free radical that can interact with different antioxidant substances. For both methods, L-ascorbic acid (50 μg/ml) was used as a positive control. The extract concentrations used for the antioxidant tests were 0.1, 0.3, 0.5, and 1 mg/ml in dark conditions. Absorbance was measured at 517 nm and 734 nm in the microplate reader. All of the experiments were performed in triplicate and repeated three times to ensure reproducibility.

Determination of the polyphenol and flavonoid contents

The polyphenol and flavonoid contents of the *U. rhynchophylla* extracts were determined as previously described [23]. Flavonoid concentration was calculated using quercetin as a standard and was expressed as mg of quercetin equivalent (QE) per 100 g of dry weight. Polyphenol was expressed as mg of gallic acid equivalent (GAE) per 100 g of dry weight.

Mushroom tyrosinase activity assay

The inhibitory strength of the *U. rhynchophylla* extracts (0.1-1 mg/ml) on tyrosinase was tested as described previously with a minor modification [28]. The reaction mixture contained 120 μl of 8.3 mM L-DOPA in a phosphate buffer (0.1 M, pH 6.8), 40 μl of extract solution, 40 μl of mushroom tyrosinase (1,000 unit/ml), and kojic acid (100 μg/ml) was used as a positive control. After pre-incubation in a water bath for 10 min at 37℃, the absorbance was measured at 492 nm using an ELISA reader. Each experiment was done in triplicate.

Cell culture

B16F10 melanoma cells (ATCC CRL-6475, from the BCRC

Cell Line Bank, BCRC60031) were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, Logan, UT, U.S.) with 10% fetal bovine serum and 1% penicillin/streptomycin at 37℃ in a humidified atmosphere with 5% CO_2 in the air.

Cell viability assay

Cell viability was determined using an MTT assay [18]. B16F10 melanoma cells were incubated in 96 microplates and pretreated with 0.1-1 mg/ml of *U. rhynchophylla* extract for 24 hr. Next, they were treated with an MTT solution (5 mg/ml in a phosphate-buffered saline, PBS), and the cells were incubated at 37℃ for 4 hr. DMSO was added after the medium was discarded and the absorbance was measured at 540 nm using an ELISA reader.

Measurement of melanin content

To determine the melanin content, minor modifications were made to a previous method [21]. B16F10 cells were seeded at a density of 1×10^5 cells per well in 6-well plates and incubated for 24 hr. The cells were treated with various concentrations of *U. rhynchophylla* extract (0.1-1 mg/ml) and either with or without 200 nM α-MSH before being incubated for 48 hr. Kojic acid was used as a positive control. The cells were washed with PBS and lysed with 1 N NaOH containing 10% DMSO at 90℃ for 30 min. The melanin content was assayed using absorbance at 400 nm.

Determination of cellular tyrosinase activity

The determination of the effect of the extracts on tyrosinase activity was carried out using a method described previously with minor modifications [30]. B16F10 cells were seeded in 6-well plates (10^5 cells per well) and were first stimulated either with or without 200 nM α-MSH for 12 hr. After that, the α-MSH-stimulated cells were incubated in the *U. rhynchophylla* extracts (0.1-1 mg/ml) for an additional 24 hr at 37℃. The treated cells were washed twice using PBS and lysed with a radio immunoprecipitation assay (RIPA) buffer. The cell lysates were harvested by centrifugation at 5,000 rpm for 5 min at 4℃. The cell extracts (25 μl) were mixed with 50 μl L-DOPA solution (10 mM in a sodium phosphate buffer) and 25 μl of 50 mM sodium phosphate buffer (pH 6.8) and incubated at 37℃ for 1 hr. The absorbance was measured at 495 nm with a microplate reader.

Tyrosinase zymography

The DOPA staining assay was performed as previously reported [21]. B16F10 cells were treated for 24 hr in the absence or presence of α-MSH at various concentrations *U. rhynchophylla* extract or 100 μg/ml of kojic acid (as positive control). The cultured cells were washed twice with PBS and collected with a lysis buffer (0.1 M sodium phosphate buffer (pH 6.8), 0.1% Triton X-100). Protein content was calculated using the Bradford method with bovine serum albumin as a standard [1]. Equal amounts (55 μg) were analyzed using a 10% polyacrylamide gel electrophoresis. After the electrophoresis, the gel was rinsed twice with 0.1 M sodium phosphate buffer (pH 6.8) with gentle shaking for 30 min and then incubated in the dark at 37℃ overnight with 5 mM L-DOPA in 0.1 M sodium phosphate buffer. Tyrosinase activity was visible in the gel as a dark melanin-containing band.

Quantitative RT-PCR analysis

RT-PCR was used to analyze the gene expression in the B16F10 mouse melanoma cells after stimulation with α-MSH in the presence of a test sample for 48 hr. B16F10 mouse melanoma cells (2×10^5 cells) were treated in the same way as described above. The total RNA was isolated with a Trizol reagent in accordance with the manufacturer's instructions (Invitrogen, Carlsbad, CA, U.S.). First-strand cDNA was synthesized from the total RNA (2 μg) containing oligo (dT) primers, and PCR amplification was performed using the Gene Amp Kit (Bioneer Corp, Korea). The primer sequences used for the PCR were (1) GAPDH (upstream 5′-GTGAAGG TCGGTGTGAACG-3′, downstream 5′-CTCGCTCCTGGAA GATGGTG-3′) and (2) tyrosinase (upstream 5′-AACAATG TCCCAAGTACAGG-3′, downstream 5′-TGACTCTTGGAG GTAGCTGT-3′). The PCR reaction involved an initial 5 min denaturation at 94℃, followed by 25 cycles at 94℃, 30 sec; 55-60℃, 30 sec; 72℃, 1 min, and a final 7 min extension. The sizes of the amplified gene products were 223 bp for GAPDH and 250 bp for tyrosinase. Aliquots of individual PCR products were separated on a 2% agarose gel, stained with ethidium bromide, and imaged by a Mupid-2plus Submarine electrophoresis system. A densitometric analysis was done using image analysis software (Gel Quant, Sam Bo Scientific Co., Ltd.).

Results

Antioxidant properties of the *U. rhynchophylla* extracts

Two assays were performed to evaluate the antioxidant characteristics of *U. rhynchophylla* extracts as shown in Fig.

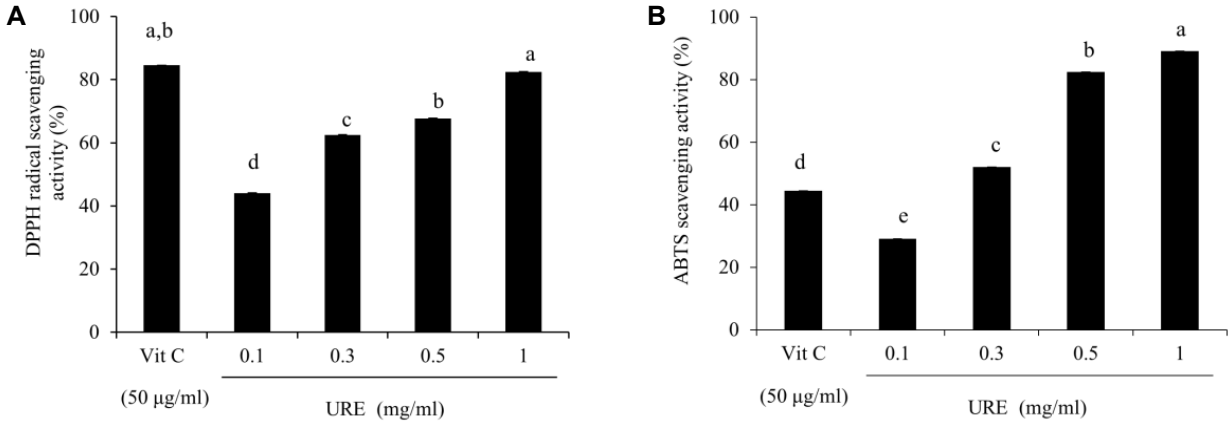

Fig. 1. The antioxidant properties of *U. rhynchophylla*. (A) DPPH free radical scavenging activity; (B) ABTS radical scavenging capacity. Various concentrations of the *U. rhynchophylla* extracts and vitamin C were as a positive standard in the above assays. URE, 70% ethanolic extract of *U. rhynchophylla*. All values are expressed as mean ± S.D. (n=3). The letters a, b, c, d, and e above the bars stand for significantly different (p<0.05) groups by one-way ANOVA, followed by Duncan's multiple test.

1. Free radical scavenging activity was determined by DPPH assay using vitamin C as a positive control. As can be seen from Fig. 1A, great variations exist in the DPPH scavenging capacity of the different extract concentrations of *U. rhynchophylla* (0.1-1 mg/ml). Likewise, the assay relies on the generation of $ABTS^{+}$ chromophores by oxidation of ABTS with potassium persulfate. For the ABTS radical scavenging assay, the *U. rhynchophylla* extract concentration of 0.3 mg/ml showed a higher ABTS scavenging capacity than that of vitamin C (50 μg/ml) (Fig. 1B).

Evaluation of the polyphenol and Flavonoid contents of *U. rhynchophylla*

The amount of total polyphenol was determined using the Folin-Ciocalteu reagent as previously described [7]. First, a standard curve was made using gallic acid as a comparative control. Fig. 2 illustrates the varying amounts of polyphenol and flavonoids in the *U. rhynchophylla* extracts. We found that with increasing concentration of *U. rhynchophylla*, there is a proportional increase in polyphenol content (Fig. 2A), and the highest concentration of *U. rhynchophylla* (1 mg/ml) has high flavonoid content (Fig. 2B). Polyphenol is one of the important plant compounds with an antioxidant capacity, probably due to its redox properties [14], which can play a major role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides.

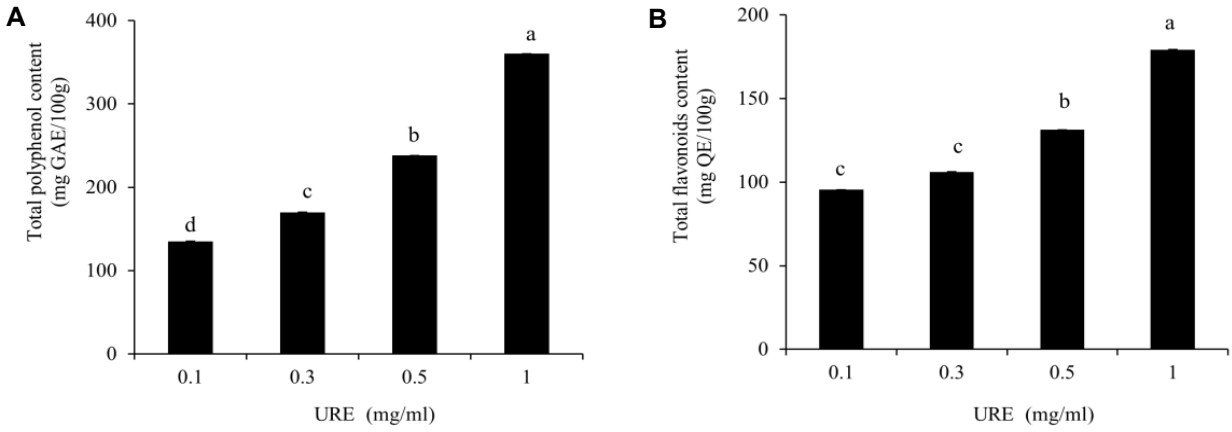

Fig. 2. Polyphenol and flavonoids contents of *U. rhynchophylla*. (A) Measurement of polyphenol contents; (B) Evaluation of flavonoid contents. URE, 70% ethanolic extract of *U. rhynchophylla*. All values are expressed as mean ± S.D. (n=3). The letters a, b, c, and d above the bars stand for significantly different (p<0.05) groups by one-way ANOVA, followed by Duncan's multiple test.

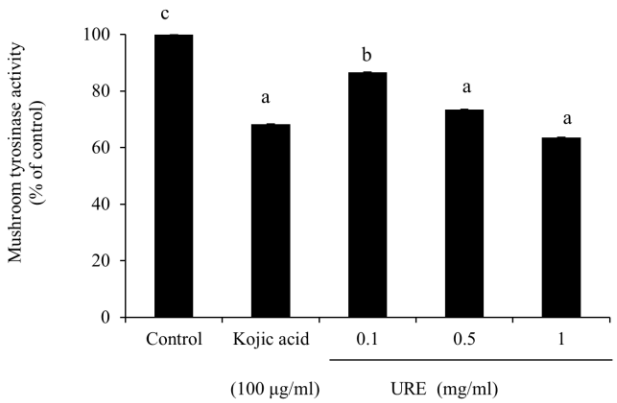

Fig. 3. Effect of *U. rhynchophylla* on mushroom tyrosinase activity. URE, 70% ethanolic extract of *U. rhynchophylla*. All values are expressed as mean ± S.D. (n=3). The letters a, b, and c above the bars stand for significantly different (p<0.05) groups by one-way ANOVA, followed by Duncan's multiple test.

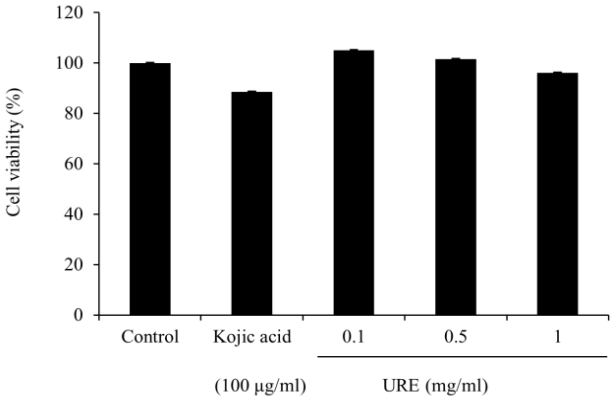

Fig. 4. Effect of *U. rhynchophylla* on cell viability in B16F10 cells. The cells were seeded at 6×10^3 cells per well and incubated in media containing 0.1-1 mg/ml concentration of URE for 24 hr. URE, 70% ethanolic extract of *U. rhynchophylla*. All values are expressed as mean ± S.D. (n=3).

Effect of *U. rhynchophylla* on mushroom tyrosinase activity

To measure the potential inhibitory effect of the *U. rhynchophylla* extracts on mushroom tyrosinase activity, enzyme inhibition experiments were carried out in triplicate. It was found that tyrosinase activity was decreased by the *U. rhynchophylla* extracts. Mushroom tyrosinase is regularly used to screen potential inhibitors of melanogenesis in experiments done *in vitro*. The 1 mg/ml concentration of *U. rhynchophylla* inhibited more of the enzyme activity than kojic acid as shown in Fig. 3. Thus, *U. rhynchophylla* seems to exhibit potent inhibition of mushroom tyrosinase activity.

Effect of *U. rhynchophylla* on cell viability

The effect of the *U. rhynchophylla* extracts on cell viability was assessed using an MTT assay. MTT is a pale-yellow compound that can be converted into a dark-blue formazan product of living cells, but dead cells do not react and remain yellow. Thus, after an MTT treatment, live cells will appear blue and dead cells will appear yellow. Following this method, it was found that the *U. rhynchophylla* extracts did not show any cytotoxicity against B16F10 cells and the cell viability remained at a similar level across all concentration levels. Even at higher concentrations, *U. rhynchophylla* showed less cytotoxicity than kojic acid as shown in Fig. 4. These results suggest that cell viability is not affected by *U. rhynchophylla* (Fig. 4), and these extracts are not responsible for any cytotoxic effects on B16F10 melanoma cell viability.

Effect of *U. rhynchophylla* on melanin content

To analyze the melanogenesis effects of the *U. rhynchophylla* extracts, the levels of melanin content were measured in both α-MSH-untreated and α-MSH-treated B16F10 cells. B16F10 cells are widely used to evaluate the anti-melanogenic effect of test materials because they share most of the melanogenic mechanisms of normal human melanocytes. The cells were pretreated either with or without α-MSH for 12 hr, followed by treatment with extracts of *U. rhynchophylla* at concentrations of 0.1-1 mg/ml or with 100 μg/ml kojic acid for an additional 24 hr. As shown in Fig. 5A and Fig. 5B, the *U. rhynchophylla* extract at a concentration of 1 mg/ml was found to significantly decrease the cellular melanin content. Moreover, the α-MSH treatment alone enhanced melanin content compared to the control, whereas the *U. rhynchophylla* extract pretreatment significantly inhibited melanin synthesis of the α-MSH induced pigmentation in a dose-dependent manner.

Effect of *U. rhynchophylla* on cellular tyrosinase activity

Since tyrosinase plays a key role in melanogenesis, we examined the inhibitory effects of the *U. rhynchophylla* extracts on cellular tyrosinase in B16F10 cells. Kojic acid, well known as an inhibitor of melanin synthesis, was used as a positive standard in this study. The results indicate that the tyrosinase activity in the B16F10 cells was inhibited by various concentrations of *U. rhynchophylla* extracts (0.1-1 mg/ml). Compared to the kojic acid, which inhibited the

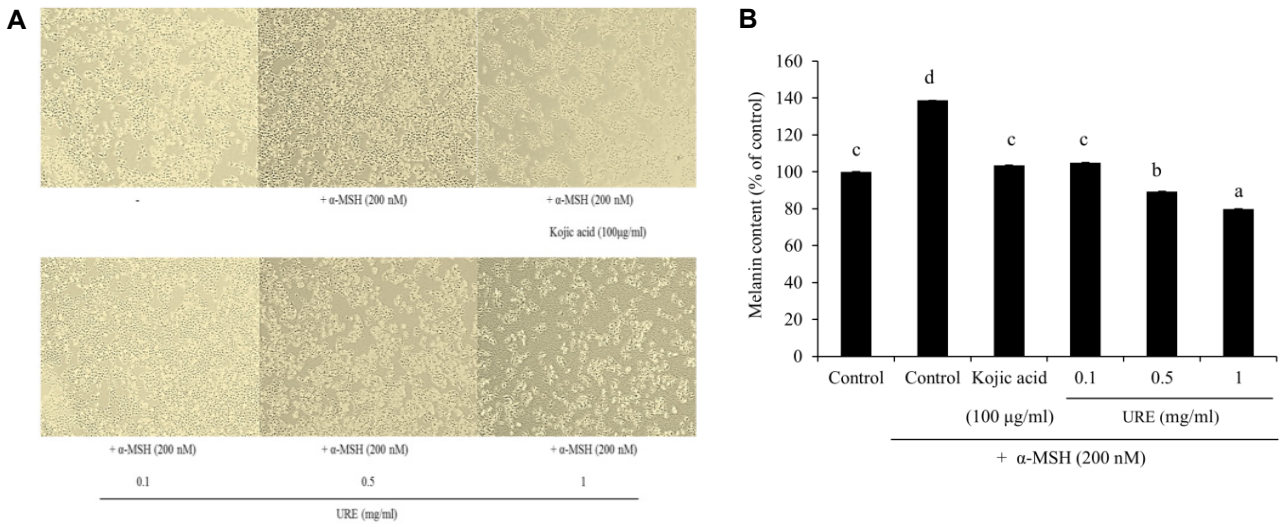

Fig. 5. Effect of *U. rhynchophylla* on melanin content. (A) Cell morphology was observed under a phase contrast microscope. (B) Effect of *U. rhynchophylla* on melanin content in B16F10 cells. The cells were treated with 0.1-1 mg/ml of URE or kojic acid (positive control) for 24 hr after treatment with/without 200 nM α-MSH for 4 hr. URE, 70% ethanolic extract of *U. rhynchophylla*. All values are expressed as mean ± S.D. (n=3). The letters a, b, c, and d above the bars stand for significantly different (p<0.05) groups by one-way ANOVA, followed by Duncan's multiple test.

tyrosinase activity as expected, *U. rhynchophylla* showed a similar tyrosinase inhibition activity at the 1 mg/ml concentration. Increasing the concentration of the extract in the reaction mixture increased the inhibitory activity of the enzyme (Fig. 6).

Effect of *U. rhynchophylla* on tyrosinase zymography

The effect of the *U. rhynchophylla* extracts on the amount of cellular tyrosinase protein was evaluated using a gel staining of tyrosinase activity (zymography) analysis. The B16F10 cells were treated with α-MSH alone or with α-MSH plus treatments (*U. rhynchophylla* extracts or kojic acid), and the total proteins in the cells were separated by gel electrophoresis and stained with L-DOPA. As shown in Fig. 6, the tyrosinase activity in the untreated cells was very light while the α-MSH-treated cells displayed a dark band with higher activity. The *U. rhynchophylla* extracts seemed to become more effective with increasing concentration, as the activity of the tyrosinase was reduced and lower bands were observed. This demonstrates that *U. rhynchophylla* extracts can significantly reduce the amount of tyrosinase protein in cells, which means that *U. rhynchophylla* extracts appear to inhibit melanogenesis of B16F10 cells by decreasing the cellular tyrosinase content and, hence, its activity.

Effect of *U. rhynchophylla* on the mRNA expression of tyrosinase

To confirm the effect of the *U. rhynchophylla* extracts on cellular melanin production, we assessed the mRNA levels

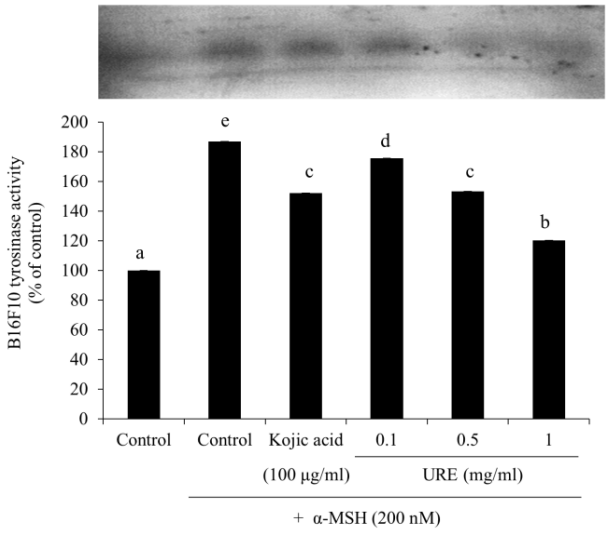

Fig. 6. Effect of *U. rhynchophylla* on cellular tyrosinase activity. The cells were treated with 0.1-1 mg/ml of URE or kojic acid as positive control for 24 hr after treatment with/without 200 nM α-MSH for 4 hr. The supernatant lysed in RIPA buffer was incubated with 5 mM L-DOPA for 1 hr and each sample was normalized to total celluar protein. URE, 70% ethanolic extract of *U. rhynchophylla*. All values are expressed as mean ± S.D. (n=3). The letters a, b, c, d, and e above the bars stand for significantly different (p<0.05) groups by one-way ANOVA, followed by Duncan's multiple test.

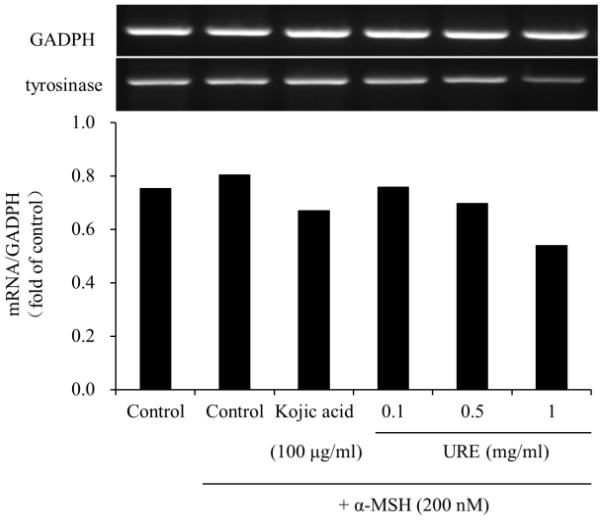

Fig. 7. Effect of *U. rhynchophylla* on the mRNA expression of tyrosinase. Total RNA from B16F10 cells treated with *U. rhynchophylla* after 200 nM α-MSH collected at the indicated time. URE, 70% ethanolic extract of *U. rhynchophylla*. Total mRNA levels were examined by RT-PCR, using glyceraldehyde 3-phosphate dehydrogenase (GADPH) as an internal control. The graph represents the fold value of exposed band intensity.

of tyrosinase along with the control GAPDH in cells treated with α-MSH and *U. rhynchophylla* extracts. As expected, the cells that had the α-MSH and *U. rhynchophylla* treatments showed increased levels of tyrosinase (Fig. 7). While lower concentrations of *U. rhynchophylla* tended to decrease tyrosinase mRNA levels, higher concentrations of *U. rhynchophylla* significantly inhibited mRNA levels. It appears that cells treated with *U. rhynchophylla* extracts show a dramatic reduction in the expression of tyrosinase mRNA levels.

Discussion

This research has identified a helpful and potential anti-melanogenic agent from a natural source. Although kojic acid is a common skin-whitening product, there is a need to find safer and more effective skin-lightening agents due to the skin irritation, low stability, and even carcinogenic possibility of kojic acid [11, 20]. This is the first report on the effect of *U. rhynchophylla* on melanin production.

In this study, it was determined that *U. rhynchophylla* extracts have a high scavenging activity, which could be attributed mainly to their levels of polyphenols and flavonoids. Tyrosinase is a multifunctional copper-containing enzyme that plays a key role in the melanin pathway and is also known as monophenol monooxygenase or polyphenol oxidase [3]. Tyrosinase catalyzes the oxidations of both monophenols and o-diphenols into reactive o-quinones. Tyrosinase contains two copper ions, which bind with histidine residues in the active site and are critical for the catalytic activity [3]. Mushroom tyrosinase is widely used as the target enzyme for screening probable inhibitors of melanogenesis. As can be seen in Fig. 3, *U. rhynchophylla* extracts displayed a higher inhibitory effect on mushroom tyrosinase activity than kojic acid. To clarify, the specific inhibitory effect of the *U. rhynchophylla* extracts on melanogenesis, B16F10 melanin content, and intracellular tyrosinase activity were evaluated. Melanin is formed from L-tyrosine in the melanosomes and is responsible for skin color. Although melanin plays a protective role against ultraviolet light and stress, the overproduction and accumulation of melanin results in hyperpigmentation and various skin problems, including age spots, freckles, and melisma [25, 29], which makes melanogenesis inhibition a matter of interest. The *U. rhynchophylla* extracts had a stronger inhibitory effect on melanin formation in B16F10 cells than kojic acid, as shown in Fig. 5B. The cell morphology was observed under a phase contrast microscope, which revealed that the *U. rhynchophylla* extracts decreased the melanin content (Fig. 5A). It has been reported that α-MSH can bind the melanocortin 1 receptor (MC1R) on the surface of melanocytes; this binding leads to the activation of adenylate cyclase, which leads to an elevated level of intracellular cAMP (cyclic adenosine monophosphate) [19]. In the study, α-MSH was used as a cAMP inducer to stimulate melanin synthesis. Research into molecules that inhibit tyrosinase has become increasingly important for cosmetic products that may be used as stronger skin-whitening agents for treating skin disorders. As shown in Fig. 6, *U. rhynchophylla* extracts were able to repress melanogenesis induced by α-MSH mediated intracellular cAMP up-regulation. To determine whether the inhibition of melanin synthesis by *U. rhynchophylla* was associated with melanogenesis-related gene expression, a RT-PCR reaction was performed to observe the mRNA levels of tyrosinase. As shown in Fig. 7, the mRNA levels of the genes tended to be reduced by the *U. rhynchophylla* extracts in a concentration-dependent manner. It is notable that the inhibition levels of the *U. rhynchophylla* extracts at higher concentrations were superior to kojic acid, which is a renowned skin whitening factor in functional cosmetics. Based on these results, *U. rhynchophylla* may act as a good tyrosinase inhibitor.

In conclusion, *U. rhynchophylla* showed inhibitory effects on melanogenesis similar to kojic acid with no adverse skin reactions such as burning, pruritus, or erythema. These findings indicate that *U. rhynchophylla* may be a useful source of bioactive compounds for anti-hyperpigmentation and as an antioxidant agent in human skincare products. In future studies, we plan to investigate the effects of various protein kinase inhibitors on melanin production in B16F10 melanoma cells after treatment with *U. rhynchophylla* and further analyze the potential active components in *U. rhynchophylla* to identify the possible skin-whitening mechanisms.

Acknowledgment

This study was supported by the Brain Busan 21+ project (BB21+).

The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : B16F10세포에서 멜라닌 생성 억제제 및 항산화제로서 조구등의 억제 효과

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최근 모든 연령대의 사람들은 미용적인 이유로 더 밝은 피부를 원하고 있으며, 천연 제품은 화학적으로 합성된 화합물보다 더 많은 관심을 받고 있다. 조구등은 아시아에서 전통 한약재로 널리 사용되어 왔다. 새로운 피부 미백제를 찾기 위해 본 연구에서는 조구등의 항산화 활성과 잠재적인 tyrosinase 억제 작용을 확인하였다. 조구등을 70% 에탄올로 추출하여 항산화 활성을 분석하고 tyrosinase 활성과 melanin 합성에 미치는 영향을 평가했다. 총 mRNA 발현은 RT-PCR을 사용하였다. 그 결과 조구등 추출물은 B16F10 세포에서 뛰어난 항산화 능력과 상당한 수준의 폴리페놀 및 플라보노이드 화합물을 함유하였다. 또한, 세포 내 tyrosinase 활성을 억제하고 처리된 세포에서 melanin의 양을 감소시켰다. Tyrosinase의 mRNA 발현은 1 mg/ml 농도에서 현저히 감소하였다. 이와 같은 결과는 조구등이 미백 효과가 있는 화장품의 천연 소재로 사용될 수 있는 높은 잠재력을 가지고 있음을 시사한다.