Inhibitory effects of *Cyrtomium fortunei J. Smith* root extract on melanogenesis

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ABSTRACT

Background: Recently, a great deal of attention has been directed toward the use of crude extracts from natural products for cosmetic applications. Thus, we performed a series of experiments to investigate skin depigmenting properties of a crude extract that was derived from a traditional Korean medicinal plant. **Materials and Methods:** In this study, the depigmentational potency of *Cyrtomium fortunei J. Smith* was investigated. The inhibitory effects of the root of *Cyrtomium fortunei J. Smith* extract on melanin production were evaluated by assessing its tyrosinase inhibitory effects, melanin production-inhibitory properties in melan-a cells and depigmenting ability in brown guinea pig skin. **Results:** The methanolic extract of the root of *Cyrtomium fortunei J. Smith* appeared to inhibit tyrosinase activity and melanin production in melan-a cells. In addition, this extract exhibited depigmenting ability on Ultra violet-induced hyper pigmentation in brown guinea pig skin. **Conclusion:** These results suggested that root of *Cyrtomium fortunei J. Smith* might prove useful in treating skin hyperpigmentation associated with excess sun-exposure.

Key words: *Cyrtomium fortunei J. Smith*, melanin, tyrosinase

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INTRODUCTION

Melanogenesis in the skin constitutes the primary cause for skin darkening. Melanin performs a valuable function by protecting the skin against ultraviolet radiation.[1] However, melanin over-production can be a serious problem, and can result in melasma, freckles, and solar (senile) purpura.[2] Melanin biosynthesis is carried out in melanocytes, and is known to be enzymetically regulated by the enzyme tyrosinase.[3] Tyrosinasecatalyzes the oxidation of tyrosine and L-dopa in the melanizationpathway[4] and is generally regarded as the key enzyme in melanogenesis.

Recently, a great deal of attention has been directed toward the use of crude extracts from natural products in cosmetic applications. Thus, we performed a series of experiments to investigate skin depigmenting properties of a crude extract that was derived from a traditional Korean medicinal plant. *Cyrtomium fortunei J. Smith* (*C. fortunei*) is an herb that has been used in Korean traditional medicine for its reputed effects as a countertoxinand hemostatic agent.[5] However, there have been very few reports on the biological effects and ingredients of this plant.[6] To the best of our knowledge, this is the first study that examined the effects of the *C. fortunei* root extract (RCE) on melaninbiosynthesis.

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MATERIALS AND METHODS

Instrumentation

A microplate reader (Molecular Devices E09090, USA) was used to measure cell viabilities, melanin content and enzyme activity. The degree of skin pigmentation was measured using a chromameter (Minolta CR-300, Japan).

Chemicals

The L-dopa, mushroomtyrosinase, kojic acid, and TPA (Phobol 12-myristate 13-acetate) used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA). The RPMI (Roswell Park Memorial Institute) medium, fetal bovine serum and antibiotic-antimycotic solution were acquired from GIBCO-BRL (Grand Island, NY, USA). All other chemicals and solvents used in this study were of analytical grade.

Plant Material and Extraction

The *Cyrtomium fortunei J. Smith* (*C. fortunei*) employed in this study was collected in June, 2007 from Kyungnam, Korea. The dried root of *C. fortunei* (100 g) was ground and extracted with methanol at 50°C, with stirring. The filtered methanol extracts were then evaporated *in vacuo*at

45°C to give 1.91 g of residue. The residue was stored at -70°C prior to use as a test sample (RCE).

Extraction of tyrosinase from Melan-A cells

Melan -a cells were disrupted via resuspensionin a tyrosinase buffer (80 mM PO_4 buffer + 1% Triton-X100 + 100 mg/ml PMSF), followed by sonication in an ice bath. After 15 minutes of centrifugation at 12,500 rpm, the supernatants were utilized in the tyrosinase assays. 150 mg of proteins were required for each of the reactions.[7]

Tyrosinase activity assay methods

The dopa-oxidase activity of tyrosinase was spectrophotometrically determined as described previously, with minor modifications.[8] Each concentration of the test substance was dissolved in MeOH. 120 ml of L-dopa (8 mM, dissolved in 67 mM phosphate buffer, pH 6.8) and 40 ml of either the same buffer or of the test sample, were added to a 96-well microplate, after which 40 ml of mushroom tyrosinase (125 U) or melan-a cell tyrosinase (150 mg)were added. After 30 minutes of incubation at 37°C, the quantity of dopachrome in the reaction mixture was determined based onoptical density at a wavelength of 492 nm (OD 492). Kojic acid was used as a reference agent.[9]

Cell line and culture procedures

In order to evaluate the effects of RCE on melanocytes, we utilized a melan-a cell model. A pigmented melanocyte cell line, 'melan-a', was previously derived from the normal epidermal melanoblasts of embryos of inbred C57BL mice.[10] The melan-a cells were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) in 200 nM TPA. 10 ml of the medium was added to a 100 mm culture dish, and the cells were seeded at a density of approximately 5×10^5 cell/dish. The cells were grown toconfluency, seeded at a concentration of 10^5 cells/well on 24-well plates, then incubated for an additional 24 hours. Each well received a daily exchange of 990 ml of medium and was treated with 10 ml of the sample for three days.

Determination of cell viabilities

The percentage of viable cells was determined by staining the cell population with crystal violet. After the media was removed from each of the wells, the cells were washed with PBS. 200 ml of crystal violet (CV 0.1%, 10% EtOH, the rest is PBS) was then added. This was incubated at room temperature for 5 minutes and washed twice in water. After the addition of 1 ml of EtOH, the samples were shaken at room temperature. The UV absorption of the resultant supernatant was measured at a wavelength of 590 nm.

Determination of melanin contents in cells

After the media was removed from each of the wells, the wells were washed in PBS. After this step, 1 ml of 1 N NaOH was added in order to dissolve the melanin. UV absorption was then measured at 400 nm, and the melanin content per well was calculated.

Measurement of depigmenting activities on brown guinea pig skin

Brown guinea pig skin is an excellent pigmentation model because its pigmentation system is resemble to human skin.[11,12] Three female brown guinea pigs weighing approximately 450 g were used in this study. The animals were purchased from OrientBio Inc., at Seoul, Korea. They were housed in an environment of constant temperature, humidity and light/dark cycle. UVB-induced hyper pigmentation elicited on the shaven backs of the animals. 500 mJ/$cm^2$of UV-B was exposed on separated areas of the back once a week three times. Test samples or vehicle (70% ethanol) were then topically applied to the hyper pigmented areas (10 ml/circle) once a day for 8 weeks.[13,14]

Statistical analysis

The data was expressed as the means ± S.E. Statistical significance was evaluated via one-way ANOVA.

RESULTS

Tyrosinase Inhibitory effect

The RC Einhibited melan-a and mushroom tyrosinase activity by 49% and 52%, respectively, at 500 mg/ml [Figure 1]. Although this inhibitory effect was lower than that of kojic acid at identical concentrations, the extract produced a graded dose response.

Inhibitory effect on melanin biosynthesis in Melan-A cells

The results of the inhibitory experiments on melanin biosynthesis in melan-a cells are presented in [Table 1]. Although RCE induced a slight decrease (4.9%) in cell viability, treatment with 100 μg/ml of RCE resulted in a 20.6% reduction in melanin production in the melan-a cells.

Depigmenting effect on hyper pigmented brown guinea pig skin

Figure 2 shows the depigmenting activity of RCE in the hyper pigmented brown guinea pig skin. Hyper pigmentation was elicited on the dorsal skin of the brown guinea pigs using UV-B radiation. UV-B irradiation induces the tanning or burning of skin.[15,16] After treatment with 3% RCE for 6 weeks, although DL-value was not statistically significant, a visible decrease in hyper pigmentation was observed when compared to the vehicle treat group. During all experimental days, visible erythema was not observed on the dorsal skin treated with RCE.

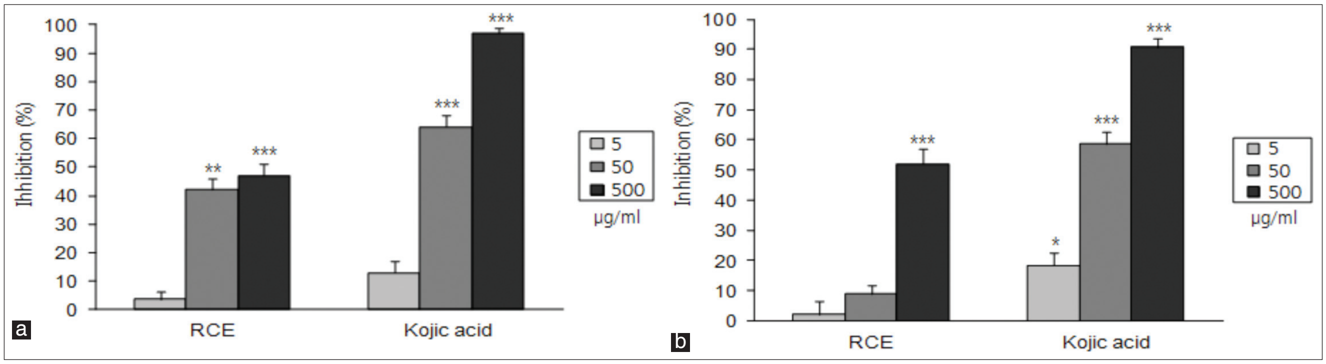

Figure 1: The effects of RCE on tyrosinase activity (a) Inhibitory effects on melan-a cell tyrosinase (b) Inhibitory effects on mushroom tyrosinase, Each value represents the mean ± S.E. of three experiments,**P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared to vehicle

Table 1: Effects of RCE on cell growth and melanin production in melan-a cells

Samples	Concentrations (μg/ml)	Melanin production (%)	Cell viability (%)
RCE	1	102.3 ± 5.0	99.9 ± 2.3
	10	92.7 ± 4.2	95.5 ± 5.1
	100	81.7 ± 6.0*	95.2 ± 5.9
Kojic acid	1	107.1 ± 9.9	96.6 ± 7.5
	10	96.0 ±2.6	98.6 ± 6.4
	100	91.9 ± 4.0	84.3 ±5.8*
PTU	1	88.9 ± 7.6	97.3 ± 1.3
(Phenyl	10	41.4 ± 9.3***	80.1 ± 9.3*
thiourea)	100	25.3 ± 8.4***	72.3 ± 9.4**

Kojic acid and PTU were used as reference materials. Each value is representative of the mean ± S.E. of three experiments, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared to vehicle

DISCUSSION

The inhibitory effects of the root of RCE on melanin production were evaluated by assessing its tyrosinase inhibitory effects, melanin production-inhibitory properties in melan-a cells and depigmenting ability in brown guinea pig skin. Overall, the results of the present study revealed that the root of *C. fortunei* inhibits melanogenesis. RCE was shown to inhibit tyrosinase activity. In melan-a cells, the melanin inhibitory effect of RCE was lower than that of Phenyl thiourea (PTU), but cell toxicity of RCE was vastly lower at same concentrations [Table 1]. In addition, RCE treatment reduced the melanin content in brown guinea pig skin. Based on these results, RCE can be expected to inhibit melanogenesis. The results of the present study indicate that RCE may prove useful as a skin depigmenting material. Furthermore, the root of *C. fortunei* might prove to be a new valuable herbal source for the development of skin depigmenting agents.

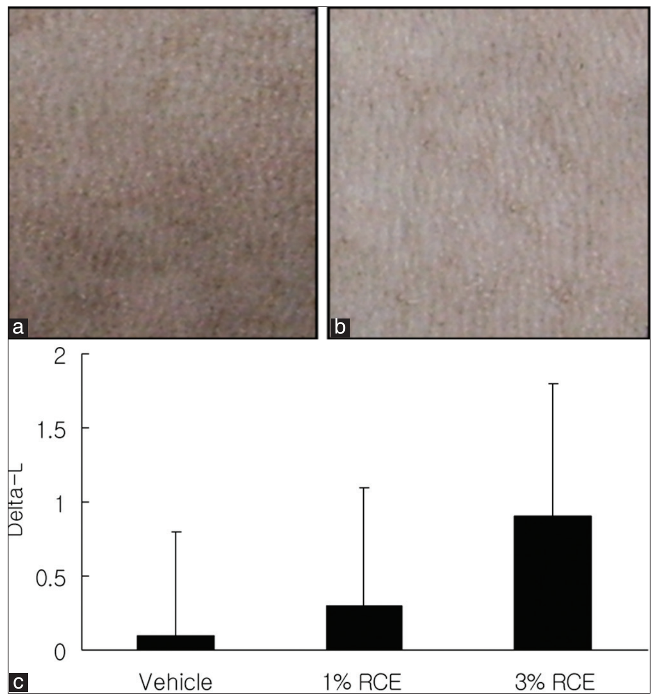

Figure 2: Depigmenting effects of RCE on UVB-induced hyperpigmentation in brown guinea pigs skin. Photographs showing changes in hyper pigmented guinea pig skin treated with vehicle alone (a) or 3% RCE in vehicle (b) after six weeks. The degree of the pigmentation decrease was represented as the DL-value (c)

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