

녹나무 부탄올 분획물이 멜라닌 생합성에 미치는 영향

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Effect of the BuOH Soluble Fraction of *Cinnamomum camphora* on Melanin Biosynthesis

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ABSTRACT : This study was carried out to investigate the effect of *Cinnamomum camphora* on melanogenesis. The MeOH extract of *Cinnamomum camphora* inhibited mushroom tyrosinase activity in dose-dependent manner. Moreover, it significantly suppressed the melanin production in melan-a cells at the concentration of 100 $\mu\text{g}/\text{ml}$. The MeOH extract was partitioned with ethyl acetate, *n*-butanol and water. Among them, the BuOH soluble fraction exhibited significant inhibitory effect on mushroom tyrosinase. In addition, the BuOH soluble fraction reduced the melanin production in melan-a cells. But, the BuOH soluble fraction had less inhibition effects on melan-a cell originated tyrosinase. So, it was performed western blotting for melanogenic proteins (tyrosinase, tyrosinase-related protein (TRP-2)) using melan-a cells. The BuOH soluble fraction inhibited the protein expression of tyrosinase at the concentration of 100 $\mu\text{g}/\text{ml}$. The results suggested that the BuOH soluble fraction of *C. camphora* might be a potent inhibitor of melanin biosynthesis in melan-a cells.

Key Words : Melanin, Tyrosinase, *Cinnamomum camphora*

INTRODUCTION

Pigmentation in mammals results from the synthesis and distribution of melanin which plays an important role in prevention of sun-induced skin injuries (Sulaimon and Kitchell, 2003). In the skin, melanocytes are occasionally present in the dermis and the basal layer of the epidermis. Synthesis of melanin in melanocytes takes place within highly specialized membrane bound intracellular organelles called melanosomes. In humans, the pigmentation of skin and hair is dependent on

size, number and distribution of the melanosomes as well as on the chemical nature of the melanins contained. Melanin is composed of two types of pigment, eumelanins (brown to black) and pheomelanins (red to yellow), and typically there are mixtures of both types in mammals (Ozeki *et al.*, 1996). Melanin production can be regulated by many biologically relevant natural agents, including interleukins, vitamins, prostaglandins, interferons, melanocyte-stimulating hormone (MSH), and UV light (Aroca *et al.*, 1993). Three related and highly similar metalloenzymes such as tyrosinase, tyrosinase-related protein

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1 (TRP-1) and tyrosinase-related protein 2 (TRP-2) are involved in the catalytic control of melanogenesis. Of these three enzymes, tyrosinase is the most critical to melanogenesis. Tyrosinase catalyzes the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to DOPAquinone. Sulfhydryl compounds such as cysteine or glutathione interact non-enzymatically with DOPAquinone to form cysteinyl DOPA, which is regarded as direct precursor of pheomelanin. Thus, the concentrations of cysteine are critical of the type of melanin synthesis. In the absence of sulfhydryl compounds, DOPAquinone spontaneously undergoes cyclization to yield DOPACHrome. In this point, there are two pathways to synthesize eumelanin. In the presence of TRP-2, called DOPACHrome tautomerase (DT), it catalyzes the tautomerization of DOPACHrome into more stable intermediate called 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Then, DHICA is converted to indole-5,6-quinone-carboxylic acid by TRP-1 and finally it makes DHICA-eumelanin. Spontaneous decarboxylation of dopachrome can also occur, leading to 5,6-dihydroxyindole (DHI). Tyrosinase catalyzes the conversion of DHI to indole-5,6-quinone and it makes DHI-eumelanin (Hearing and Tsukamoto, 1991; Prota, 1993; del Marmol and Beermann, 1996).

Since the 1980s, arbutin, kojic acid, vitamin C and its derivatives were developed as whitening cosmetics and medicines but the clinical effect of these materials is unsatisfactory (Curto *et al.*, 1999). The hydroquinone group compounds have been used as effective depigmenting agents for skin over-pigmentation, but they are strongly irritable and exhibit cell toxicity (Maeda *et al.*, 1997; Curto *et al.*, 1999). Therefore, researchers are searching for a natural product that is safer and more effective inhibitor of melanin synthesis (Jo *et al.*, 2008; Jeong *et al.*, 2009). The 95 wild plants from Cheju island originated natural resources were screened. This study showed that the extract of *Cinnamomum camphora* significantly inhibits mushroom tyrosinase activity.

Cinnamomum camphora Sieb (known as camphor) is a plant of family Lauraceae, and grown mainly in Korea (especially Cheju island), Japan, Taiwan, China, and Indonesia. The fragrant components of *Cinnamomum camphora* are camphor, safrole, and cineol that are used as a drug to treat rheumatism, sprains, asthma, neurasthenia, epilepsy, cystitis, and pyelonephritis in folk remedies. In addition, it has been known as stimulants or cardiac stimulants (Mukherjee *et al.*, 1994). However, there are few reports of biological activity on these components.

In the present work, the methanol extract and the butanol soluble fraction of *Cinnamomum camphora* were investigated as a candidate material for inhibiting melanin biosynthesis in melan-a cells. This study shows that melanin production in melan-a cells treated with the BuOH soluble fraction decreases in a dose-dependant manner without cell toxicity by reducing the level of tyrosinase protein. These results further suggest that *Cinnamomum camphora* may be a candidate resource for skin lightening as well as for treating epidermal hyperpigmentation.

MATERIALS AND METHODS

Materials

Reagents

RPMI 1640, fetal bovine serum (FBS), penicillin-streptomycin (PS) were purchased from Gibco (Gland Island, NY, U.S.A.). All other chemicals, including Triton X-100, phenylmethylsulfonyl fluoride (PMSF), kojic acid, aprotinin, L-DOPA and (+)-camphor were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.). We used analytical grade solvents without further purification.

Plant materials

Cinnamomum camphora was cultivated in Cheju island, Korea. The leaves of *Cinnamomum camphora* (family of Lauraceae) were collected by Prof. Eun-Sook Yoo at Cheju University during April to May in 2004.

Methods

Gas chromatography analysis

Dry powdered leaves (13.1 g) of *Cinnamomum camphora* were extracted with hexane using an ultrasonic apparatus for 1h. The extract solution (206 mg) was then filtered and concentrated under a vacuum at 45°C. The camphor content of sample was analyzed by gas chromatography (GC). Identification of the constituent was based on comparison of the retention time with that of authentic camphor ((1R)-(+)-camphor, 98%). GC analysis was performed with a gas chromatography (GC-17A, Shimadzu, Japan) equipped with an omega wax 320 Fused Silica Capillary column (30 m × 0.32 mm, i.d. 25 μm film thickness, Supelco, Sigma aldrich Co. U.S.A.). Analytical conditions were: injector

and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60 to 240°C at 5°C/min; carrier gas helium at 1 ml/min; injection of 1 µl; split ratio 1 : 30.

Extraction and partition

Air-dried leaves of *Cinnamomum camphora* were ground into fine powder in a mill. At room temperature, dry powdered leaves of *Cinnamomum camphora* were extracted three times with 80% methanol (MeOH) using an ultrasonic apparatus for 1 h. The extract solution was filtered, taken to dryness under reduced pressure and concentrated *in vacuo* to give a MeOH extract. The MeOH extract was suspended with distilled water, and then successively partitioned with ethyl acetate (EtOAc) and water-saturated *n*-butanol (BuOH) respectively. The EtOAc, BuOH and water soluble fractions were independently evaporated under reduced pressure at 45°C and completely dried by lyophilization.

Inhibition of tyrosinase activity

Tyrosinase extraction from melan-a cells

The melan-a cells were disrupted by resuspending in a tyrosinase buffer (80 mM PO₄ buffer + 1% Triton-X 100 + 100 µg/ml PMSF) and followed by sonication in an ice bath. After centrifugation at 12,500 rpm for 30 min at 4°C, the supernatant was used for the enzyme assay. 150 µg of proteins were required for each reaction (Fuller *et al.*, 2000). Protein content was measured using the Bio-Rad Protein Assay Kit (Bio-Rad, Richmond, CA, U.S.A.) with BSA as the standard.

Inhibition of mushroom and cell originated tyrosinase activity

Tyrosinase activity was measured by determining its DOPA oxidase activity using a modification of the method reported by Shono *et al* (1981).

Each concentration (1 µg/ml, 10 µg/ml and 100 µg/ml) of the test substances was dissolved in methanol. 120 µl of L-DOPA (8 mM dissolved in 67 mM phosphate buffer, pH 6.8) and 40 µl of either the same buffer or test sample was added to each well of a 96 well plate and then 40 µl of mushroom (125 U) or cell originated tyrosinase were added. The amount of dopachrome in the reaction mixture was determined after 30 min at 37°C. Inhibitory activity was

determined by optical density at 490 nm (OD₄₉₀). Kojic acid was used as a positive control.

Cell line and culture procedures

The melan-a cells were kindly donated by Dr. Byeong Gon Lee at the Skin Research Institute, Amore-Pacific Co., Yongin city Kyunggi, Korea. The melan-a cells were cultured in RPMI1640 medium with 10% FBS and 200 nM phorbol 12-myristated-13-acetate (TPA) conditions. Cells were seeded in a 100 mm diameter tissue culture dish with approximately 5 × 10⁵ cells. Cells were grown to confluence after 3 to 4 d at 37°C in 5% CO₂, and were seeded at 10⁵ cells/well in a 24 well plate, and then incubated for a further 24 h. Each well was renewed with 990 µl of medium daily and treated with 10 µl of 1, 10 or 100 µg/ml (final concentration) of test sample for 3 d (solvent system; propylene glycol : EtOH : H₂O = 5 : 3 : 2).

Phenylthiourea (PTU), which is an inhibitor of melanogenesis acting on tyrosinase, was used as a positive control.

Melanin contents and cell viability in cultured melan-a cells

Determination of cell viability

The percentage of viable cells was determined by staining the cell population with crystal violet. After removing the media on each well, the cells were washed with PBS. The 200 µl of crystal violet (CV 0.1%, 10% EtOH, the rest is PBS) was added. It was incubated at room temperature for 5 min, and washed twice with water. After adding 1 ml of EtOH, it was shaken at room temperature for 10 min. The crystal violet absorption was measured at 590 nm.

Determination of the melanin level

The melanin content was measured using a modification of the methods reported by Hosoi *et al* (1985). After removing the media from each well, it was washed with PBS. This procedure was followed by adding 1 ml of 1 N NaOH to dissolve the melanin. The maximum absorption was measured at 400 nm, and the melanin content per well was calculated, and is expressed as a percentage of the control.

Western blot analysis

The melan-a cells were washed with PBS and harvested

by a trypsin-EDTA solution. The pellet was washed and extracted in a triple-detergent lysis buffer (50 mM Tris-Cl, pH 8.0, 0.1% SDS, 150 mM NaCl, 1% NP-40, 0.02% sodium azide, 0.5% sodium deoxycholate, 100 $\mu\text{g}/\text{mL}$ PMSF, and 1 $\mu\text{g}/\text{mL}$ aprotinin). The protein content in the supernatant was determined using a Bio-Rad Protein Assay Kit with BSA as the standard. The supernatant was boiled with a SDS-sample buffer. 50 μg of the protein was subjected to electrophoresis on 8% SDS-PAGE gel and transferred to a Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech, Buckinghamshire, UK). The membrane was blocked using 5% skim milk for 1 h at room temperature and incubated with tyrosinase (Santa Cruz, CA, USA) and TRP-2 (Santa Cruz, CA, USA) primary antibodies for overnight at 4°C. After removing the primary antibodies, the membrane was washed with the PBS buffer and then incubated with the anti-goat secondary antibodies for 1 h. The immunoreactive proteins were detected with ECL reagent (Amersham Pharmacia Biotech Buckinghamshire, UK).

Statistical analysis

Data were expressed as mean \pm S.D. from at least three independent experiments. Student's *t* test was used for statistical analyses and only *P*-values < 0.05 were reported as significant. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 indicate statistically significant differences from the control group.

RESULTS

The major compound of *Cinnamomum camphora*, (+)-camphor

To confirm the major compound of *Cinnamomum camphora*, quantitative analysis using GC was performed. The dried powdered leaves of *Cinnamomum camphora* extracts contained (+)-camphor (23.55 $\mu\text{g}/\text{g}$), as a standardized major active component, were identified as determined (data not shown). In this study, (+)-camphor was used only standard material for quantitative analysis of *Cinnamomum camphora*.

Extraction and partition from *Cinnamomum camphora*

The powdered dry leaves (550.7 g) of *Cinnamomum camphora* were extracted three times with 80% methanol for 1 h. The extract solution was evaporated under reduced pressure at 45°C to complete dryness and gained 119.1 g MeOH extract. The MeOH extract was suspended with 4 L

distilled water, and then partitioned with EtOAc (12 L). The organic layer was evaporated to give an EtOAc soluble fraction (13.4 g). The EtOAc insoluble part was extracted with water-saturated *n*-BuOH (12 L). The BuOH extracts were combined and evaporated to afford a BuOH soluble fraction (28.8 g). The aqueous layer was evaporated and then followed by lyophilization to give a water soluble fraction (Fig. 1).

Inhibitory effect of MeOH extract of *Cinnamomum camphora* on mushroom tyrosinase activity

80% of the MeOH extract of *Cinnamomum camphora* and kojic acid as a positive control were examined for the mushroom tyrosinase inhibitory activity. Each sample of the MeOH extract and kojic acid were dissolved to 1, 10, and 100 $\mu\text{g}/\text{mL}$ in MeOH. As shown in Fig. 2, the MeOH extract inhibited mushroom tyrosinase activity. However, kojic acid, which is a fungal metabolic product and well recognized for its effective inhibition of tyrosinase in cosmetics,

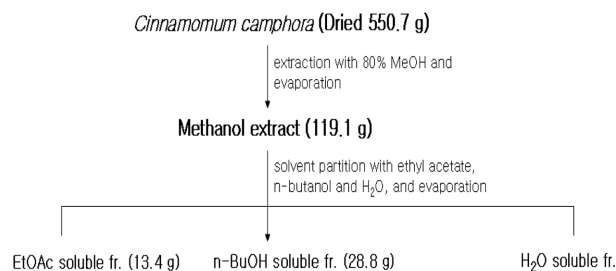


Fig. 1. Extraction and partition of fractions from *Cinnamomum camphora* leaves

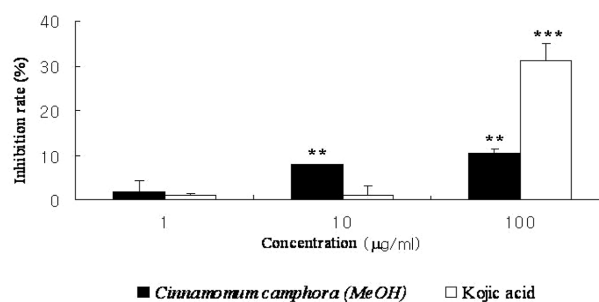


Fig. 2. Inhibitory effects of MeOH extract from *Cinnamomum camphora* and kojic acid on mushroom tyrosinase activity. Samples were dissolved in MeOH. 40 μL of mushroom tyrosinase reacted with 120 μL of L-DOPA and each sample at 37°C for 30 min. After reaction, dopachrome contents were measured at 490 nm by ELISA reader. The MeOH extract shows inhibitory effect but less than kojic acid, positive control on mushroom tyrosinase. Each value represents the mean \pm S.D. of three experiments (***P* < 0.01 and ****P* < 0.001, compared with control, vehicle treatment group).

inhibited mushroom tyrosinase activity greater than the MeOH extract at the high concentration of 100 $\mu\text{g/ml}$.

The effects of MeOH extract of *Cinnamomum camphora* on cell growth and melanin production in melan-a cells

To provide more direct evidence that MeOH extract of *Cinnamomum camphora* inhibits melanogenesis, the effects of MeOH extract on melanin production in melan-a cells were evaluated in addition to the cell toxicity. After treatment of 1 $\mu\text{g/ml}$, 10 $\mu\text{g/ml}$ and 100 $\mu\text{g/ml}$ of the MeOH extract for 72 h, the melanin content of cells was determined as shown in Fig. 3. At the concentration of 100 $\mu\text{g/ml}$, it induced 30% inhibition of melanin formation by melan-a cells as compared with non-treated cells without significant cytotoxicity. PTU as positive control showed 50% inhibition of melanin production without cytotoxicity at 10 $\mu\text{g/ml}$ but with significant cytotoxicity at 100 $\mu\text{g/ml}$.

Inhibitory effect of BuOH soluble fraction of *Cinnamomum camphora* on mushroom tyrosinase activity

Of three partitioned fractions, particularly, the BuOH

soluble fraction of *Cinnamomum camphora* has an inhibitory effect on mushroom tyrosinase activity (Table 1 and Fig. 4). Moreover, at the concentration of 1 and 10 $\mu\text{g/ml}$, the BuOH fraction inhibited more strongly than kojic acid at the same concentration.

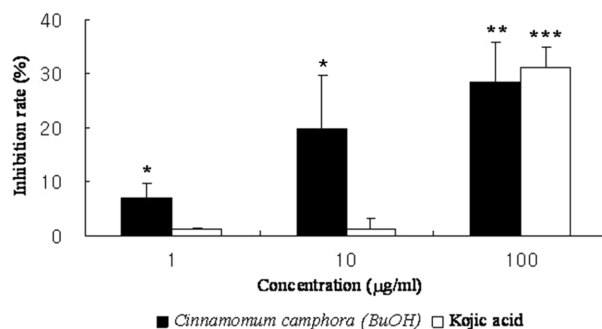


Fig. 4. Inhibitory effects of BuOH soluble fraction of *Cinnamomum camphora* and kojic acid on mushroom tyrosinase activity. Samples were dissolved in MeOH. 40 μl of mushroom tyrosinase reacted with 120 μl of L-DOPA and each sample at 37°C for 30 min. After reaction, dopachrome contents were measured at 490 nm by ELISA reader. Each value represents the mean \pm S.D. of three experiments (*P < 0.05, **P < 0.01 and ***P < 0.001, compared with control, vehicle treatment group).

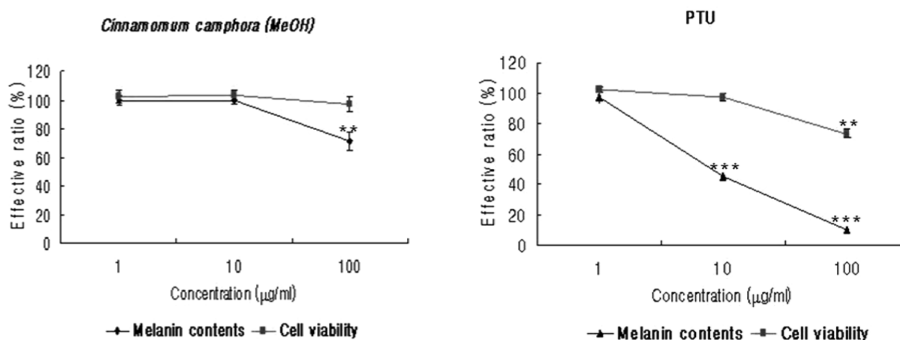


Fig. 3. The effects of MeOH extract of *Cinnamomum camphora* and PTU on cell growth and melanin production of melan-a cells. PTU is used as a positive control. Each value represents the mean \pm S.D. of three experiments (**P < 0.01 and ***P < 0.001, compared with control, vehicle treatment group).

Table 1. Inhibitory effects of BuOH soluble fraction of *Cinnamomum camphora* and kojic acid on mushroom tyrosinase activity, compared with EtOAc soluble fraction and water soluble fraction. Each value represents the mean \pm S.D. from at least three independent experiments.

Sample	Concentration ($\mu\text{g/ml}$)	Inhibition (%)	Sample	Concentration ($\mu\text{g/ml}$)	Inhibition (%)
EtOAc fr.	1	0.63 \pm 3.9	H ₂ O fr.	1	-2.47 \pm 1.2
	10	1.52 \pm 8.6		10	-2.03 \pm 0.9
	100	-10.06 \pm 1.2		100	-3.51 \pm 1.5
BuOH fr.	1	9.83 \pm 4.6	Kojic acid	1	0.49 \pm 13.7
	10	14.7 \pm 7.4		10	1.75 \pm 11.4
	100	23.88 \pm 7.2		100	37.26 \pm 12.9

The effects of BuOH soluble fraction of *Cinnamomum camphora* on cell growth and melanin production in melan-a cells

In order to investigate more direct evidence that BuOH soluble fraction of *Cinnamomum camphora* inhibits melanogenesis, its effects on melanin production in melan-a cells were determined. Melanin synthesis was significantly inhibited at 100 $\mu\text{g/ml}$ while the melanin content was reduced to 35% of that in non-treated cells at the same concentration (Fig. 5).

Inhibitory effect of BuOH soluble fraction of *Cinnamomum camphora* on melan-a cell originated tyrosinase activity

We examined the inhibitory action of the BuOH soluble fraction of *Cinnamomum camphora* on cellular tyrosinase activity in melan-a cells. As shown in Fig. 6, the inhibitory

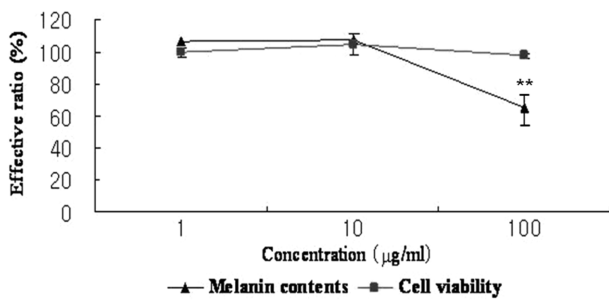


Fig. 5. The effects of BuOH soluble fraction of *Cinnamomum camphora* on cell growth and melanin production of melan-a cells. Each value represents the mean \pm S.D. of three experiments (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, compared with control, vehicle treatment group).

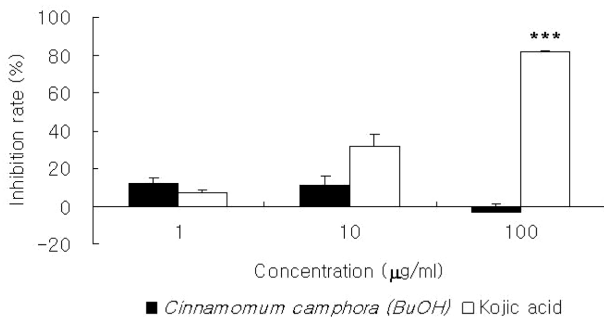


Fig. 6. Inhibitory effects of BuOH soluble fraction of *Cinnamomum camphora* on melan-a cell originated tyrosinase activity. Samples were dissolved in MeOH. 150 μg of melan-a cell originated tyrosinase reacted with 120 μl of L-DOPA and each sample at 37°C for 30 min. After reaction, dopachrome contents were measured at 490 nm by ELISA reader. Each value represents the mean \pm S.D. of three experiments (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, compared with control, vehicle treatment group).

effects of the BuOH fraction on melan-a cell tyrosinase show different appearance compared to on mushroom tyrosinase. The BuOH fraction didn't significantly inhibit melan-a cell originated tyrosinase activity but kojic acid did.

Tyrosinase and TRP-2 expression of melan-a cells treated with BuOH soluble fraction of *Cinnamomum camphora*

To determine whether these changes in tyrosinase activity could be related to a variation in the relative amount of this enzyme in melan-a cells, protein level of tyrosinase and TRP-2 was evaluated by western blot analysis. Protein was isolated from melan-a cells after treatment with the BuOH soluble fraction of *Cinnamomum camphora*. It revealed that tyrosinase protein levels were decreased by the BuOH soluble fraction, which had no effect on TRP-2 protein levels. (Fig. 7).

DISCUSSION

Melanogenesis, a major differentiated function of melanocytes, plays an important role in protecting skin from sun-related injuries and is principally responsible for skin color. Tyrosinase is the key enzyme for melanin biosynthesis and it catalyzes the first two steps in melanin synthesis: the hydroxylation of tyrosine to L-DOPA and the oxidation of L-DOPA to

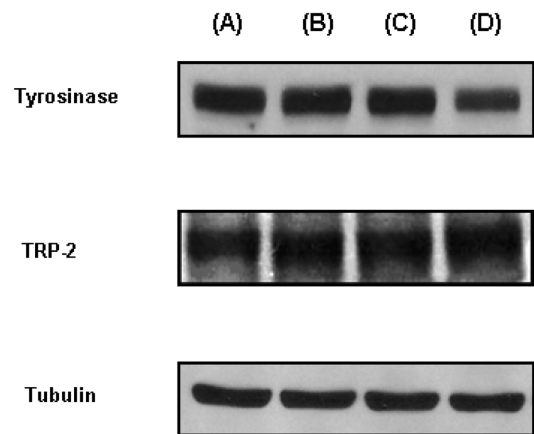


Fig. 7. The effects of BuOH soluble fraction of *Cinnamomum camphora* on tyrosinase and TRP-2 expression in melan-a cells. Control (A), 1 $\mu\text{g/ml}$ (B), 10 $\mu\text{g/ml}$ (C), and 100 $\mu\text{g/ml}$ (D) of BuOH fraction. Melan-a cells were treated with the BuOH soluble fraction for 72 h. Equal amounts of total proteins (40 $\mu\text{g/lane}$) were subjected to 8% SDS-PAGE and tyrosinase and TRP-2 protein were detected by western blotting using specific antibody. Equal protein loading was confirmed by blotting with anti-tubulin.

DOPAquinone (Hearing and Tsukamoto, 1991; Prota, 1993; del Marmol and Beermann, 1996). This process is a determinant of animal skin color, and is involved in pigmentary disorders such as melanoma, ephelides, post-inflammatory hyperpigmentation and solar lentigos (Briganti *et al.*, 2003). Several materials such as kojic acid and hydroquinone group compounds have been used as skin-lightening agents in the cosmetic industry but they are strongly irritable and exhibit cell toxicity. Because of these reasons, natural resources have attracted increasing attention as depigmenting resources.

During the screening study of wild plants from Cheju Island originated natural resources, it was found that the methanol extract obtained from leaves of *Cinnamomum camphora* showed the significant inhibitory effect on mushroom tyrosinase, as shown in Fig. 2. However, there are few reports of the biological activity as well as the melanin biosynthesis inhibitory effects of *Cinnamomum camphora* (Takaoka and Hiroi, 1976; Takaoka *et al.*, 1979; Mukherjee *et al.*, 1994).

To elucidate the effect of *Cinnamomum camphora* on melanogenesis, we performed tyrosinase enzyme activity assays, melanin assays, and western blotting for melanogenic proteins (tyrosinase, and TRP-2) using melan-a cells.

Mentioned above, the MeOH extract of *Cinnamomum camphora* inhibited mushroom tyrosinase activity in dose-dependent manner but its inhibitory effect was not much higher than kojic acid known as tyrosinase inhibitor (Fig. 2). Moreover, it significantly suppressed the melanin production in melan-a cells at the concentration of 100 $\mu\text{g}/\text{m}\ell$ (Fig. 3).

Partition of the MeOH extract gave the following three subfractions: the ethyl acetate soluble fraction, the *n*-butanol soluble fraction and the water soluble fraction (Fig. 1). Among them, the BuOH soluble fraction exhibited significant inhibitory effect on mushroom tyrosinase, whereas the EtOAc soluble fraction and the water soluble fraction were inactive (Table 1 and Fig. 4). These results show that materials having the depigmenting effect in *Cinnamomum camphora* were contained into the BuOH soluble fraction. To investigate the inhibitory effect of the BuOH soluble fraction on melanin synthesis, we treated it in melan-a cells for 3 days. Melan-a cells are synergistic with the B16 melanoma and their sublines, and provide an excellent parallel non-tumorigenic line for studying the melanoma malignancy (Bennett *et al.*, 1987). The BuOH soluble

fraction reduced the melanin production in melan-a cells (Fig. 5), but the others didn't have an effect on depigmenting effect.

Because of its simple method, mushroom tyrosinase assay is good to select materials having anti-melanogenic activity at the first stage. However, the results of it could be different with the result of the assay using cell-originated tyrosinase. Using extracted melan-a cell tyrosinase, a tyrosinase inhibition test was performed with the BuOH soluble fraction. The BuOH soluble fraction didn't show similar inhibition effects compared to mushroom tyrosinase. As shown in Fig. 6, the BuOH soluble fraction had less inhibitory effects to melan-a cell-originated tyrosinase.

This study was carried out to evaluate the protein levels of tyrosinase and TRP-2 on melan-a cells treated with the BuOH soluble fraction of *Cinnamomum camphora*. The BuOH soluble fraction affected the amount of tyrosinase protein (Fig. 7). Results showed that it did not inhibit tyrosinase enzyme activity but reduced tyrosinase protein expression. It is known that in addition to tyrosinase, there are other regulatory enzymes for melanin formation, such as TRP-2 (DT) and TRP-1. TRP-2 favors the formation of DHICA instead of DHI. DHICA-derived melanin is black and flocculent whereas DHI-derived melanin is yellowish-brown and finely dispersed (Hearing and Tsukamoto, 1991). However, no significant changes were observed for TRP-2 protein level from either treatment of the melan-a cells (Fig. 7). We did not attempt to measure the TRP-1 activity in this present experiment, although we believe that TRP-1 is a predominant and related melanogenic protein (Kobayashi *et al.*, 1994) with an unknown function.

In conclusion, this study has demonstrated that the inhibitory activity of the BuOH soluble fraction of *Cinnamomum camphora* for melanin synthesis is mediated by decreasing expression of tyrosinase in melan-a cells. In addition, the BuOH soluble fraction is the most promising one to isolate the active depigmenting components. Isolation of active component with inhibitory effects on melanogenesis from the BuOH soluble fraction of *Cinnamomum camphora* is in progress. Finally, the fractions or isolated compound from *Cinnamomum camphora* may be a useful inhibitor of melanogenesis and have beneficial effects in the treatment of hyperpigmentation disorders.

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