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Inhibitory Effects of Hydrolysable Tannins on Tyrosinase Activities in B16 Mouse Melanoma Cells

Soo Min Cho, Young Min Kwon, Jae Hee Lee, and Min Won Lee*

College of Pharmacy, Chung-Ang University, Seoul, 156-756, Korea

Abstract – To investigate skin whitening natural substances, the effects on melanogenesis by measuring the tyrosinase activity and the melanin contents of three hydrolysable tannins, 1,2,6-tri-*O*-galloyl-β-D-glucose (**1**), 2,3-(S)-HHDP-D-glucose (**2**) and pedunculagin (**3**) in B16 melanoma cells were examined. 1,2,6-Tri-*O*-galloyl-β-D-glucose (**1**), 2,3-(S)-HHDP-D-glucose (**2**) and pedunculagin (**3**) inhibited tyrosinase activity in B16 melanoma cells in a dose-dependent manner.

Key words – Hydrolysable tannin, melanogenesis inhibition

Introduction

Recently, it was known that many natural components had skin whitening effect (Chun *et al.*, 2000; Lee *et al.*, 1997) and used as cosmetic sources. It was reported that tannin had various biological activities for its enzyme inhibition (Inokuchi *et al.*, 1985; Kuramochi *et al.*, 1992; Kashiwada *et al.*, 1992) including inhibitory activities of tyrosinase (Kim *et al.*, 1997; Iwata *et al.*, 1990) and melanogenesis (Kim *et al.*, 2001). Previously, we also found inhibitory effect of tannin on tyrosinase activity (Cho *et al.*, 2001). Further studies on whitening effect of tannin, we examined the inhibitory effect of several hydrolysable tannins on tyrosinase activities and melanin biosynthesis in B16 melanoma cells.

Materials and Methods

Isolation and identification – Extraction and isolation of the compounds from fresh leaves (2.5 kg) of *A. hirsuta* Turcz var. *microphylla* (Lee *et al.*, 1992) and fruits of *Rubus coreanum* were carried out as described previously (Pang *et al.*, 1996). Full details of the isolation and characterization are available on request from the authors correspondence.

Cell culture – B16 murine melanoma cells were cultured in Dulbeccos modified Eagles medium with 10% fetal bovine serum and penicillin/streptomycin (100IU/50 μg/ml) in a humidified atmosphere containing 5% CO_2 in air at 37°C.

Enzyme activity assay and melanin determination – Tyrosinase activity was estimated by measuring the rate of oxidation of L-dopa. Cells from a subconfluent monolayer in a 24-well plate were suspended in 100 μl of phosphate buffer, pH 6.8, containing 1% (w/v) Triton X-100. After vortexing to lyse the cells, the compounds were clarified by centrifugation at 10,000 rpm for 5 min in an eppendorf tube. The tyrosinase substrate L-dopa (2 mg/ml) was prepared in the same lysis phosphate buffer (without Triton). 40 μl of each compound was put in a 96-well plate, and 100 μl of lysis buffer was added to start the enzymatic assay. Absorbance at 570 nm was read after 1 hr incubation at 37°C using a microplate reader.

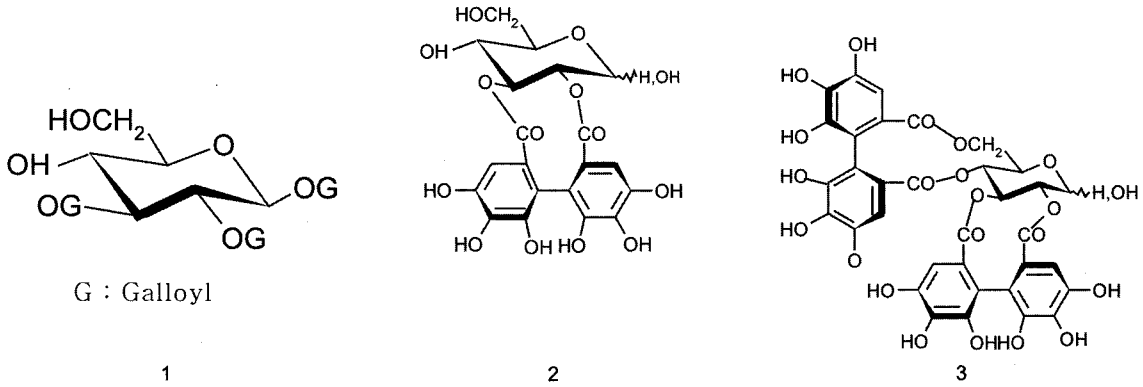
For melanin determination, after 48 hr treatment with the samples, cells from a confluent 3.5 cm diameter plate were solubilized in 100 μl of 1 N NaOH and diluted with 400 μl of distilled water. The samples were incubated at 60°C for 1 hr and vortexed to solubilized the melanin. Absorbance at 405 nm was compared with a standard curve of known concentrations of fungal melanin prepared in a final NaOH concentration of 0.2 N.

Results and Discussion

1,2,6-Tri-*O*-galloyl-β-D-glucose (**1**) was isolated from fresh leaves (2.5 kg) of *A. hirsuta* Turcz var. *microphylla*. 2,3-(S)-HHDP-D-glucose (**2**) and pedunculagin (**3**) were isolated from the fruits of *Rubus coreanum*.

MTT assay showed that non-cytotoxic range of these hydrolysable tannins on B16 melanoma cells as 0-20 μg/ml and the treatment ranges of compounds were determined as 2.5, 5, 10 and 20 μg/ml (Fig. 1).

*Author for correspondence, E-mail: mwlee@cau.ac.kr



Tyrosinase activity was reduced by addition of the hydrolysable tannins to incubation medium of the melanoma cells. All the compounds showed inhibitory effect on intrinsic tyrosinase activity, but it did not show inhibitory activity on melanin level in melanoma cells (Fig. 2-4).

These results suggest that these hydrolysable tannins are potential inhibitors against melanogenesis by inhibition of intrinsic tyrosinase activity.

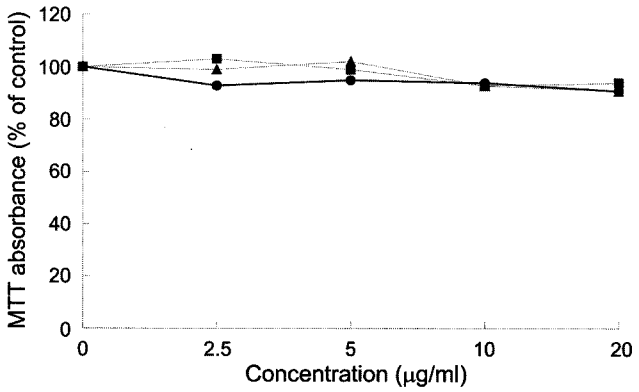


Fig. 1. Effect of compounds **1-3** on the viability of B16 melanoma cell. The viability of the cells was measured by MTT assay and result was expressed as % control.

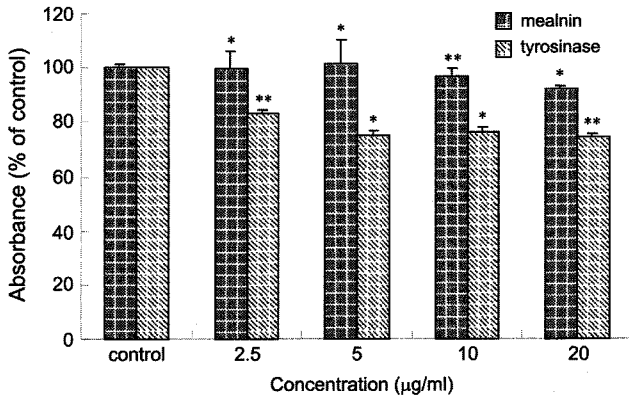


Fig. 3. Inhibitory effect of melanin synthesis and tyrosinase activity of compound **2**. Results were expressed as % control and data were mean±S.E. of at least three determination. *Significantly different from control group (**p<0.01, *p<0.05).

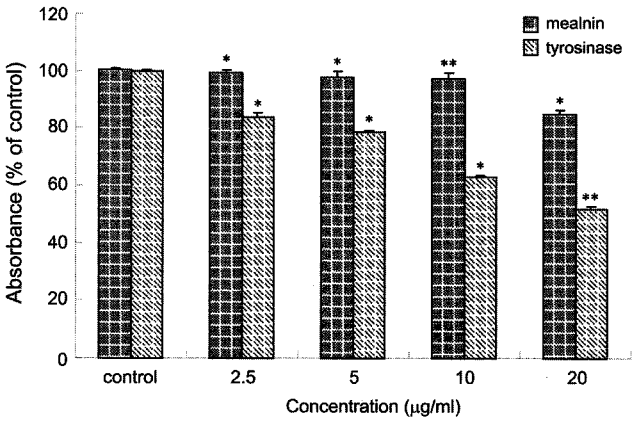


Fig. 2. Inhibitory effect of melanin synthesis and tyrosinase activity of compound 1. Results were expressed as % control and data were mean±S.E. of at least three determination. *Significantly different from control group (**p<0.01, *p<0.05).

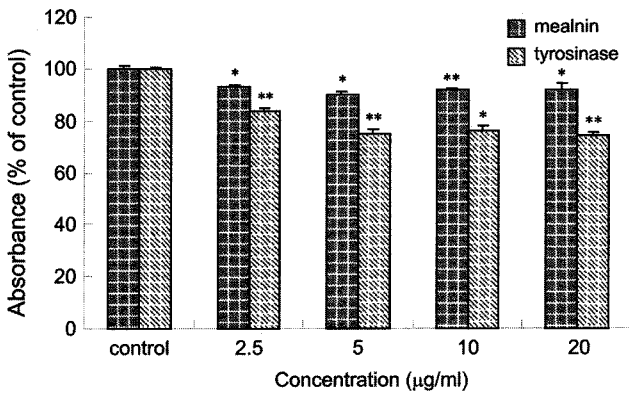


Fig. 4. Inhibitory effect of melanin synthesis and tyrosinase activity of compound **3**. Results were expressed as % control and data were mean±S.E. of at least three determination. *Significantly different from control group (**p<0.01, *p<0.05).

Acknowledgements

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