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보골지에서 분리된 Polyphenol 화합물의 멜라닌 생성억제 활성

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Tyrosinase Inhibitory Polyphenol Compounds from the Seed of *Psoralea corylifolia*

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Objectives

Tyrosinase (1.14.18.1), widely distributed in nature, is a metalloenzyme oxidase which catalyzed two distinct reactions of melanin synthesis; the hydroxylation of monophenol and the oxidation of *o*-diphenol to the corresponding *o*-quinon. Tyrosinase is not only responsible for browning of hair and skin pigmentation, but also it may play a role in cancer and neurodegenerative diseases in mammals. As, tyrosinase inhibitors have recently attracted a lot of interest due to decrease of the hyperpigmentation resulting from the enzyme action. Hence, tyrosinase inhibitors are supposed to have broad applications in medicinal and cosmetic products. Search for tyrosinase inhibitors from medicinal plants, we found that the chloroform extracts from the seed of *Psoralea corylifolia* can remarkably inhibit tyrosinase activity.

Materials and Methods

Materials

Organic solvents used for isolation were of first grade and the stock solution and buffers were prepared with milli Q water. Methanol, acetonitrile and acetic acid for HPLC were purchased as of analytical grade from J.T. Baker (Phillipsburg, NJ, USA). Column chromatography was carried out using silica gel (230-400 mesh, Merck), RP-18 (ODS-A, 12 nm, S-150 mM, YMC), and Sephadex LH-20 (Amersham Biosciences). For biochemical assays, Mushroom tyrosinase (EC 1.14.18.1), L-tyrosine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). *P.corylifolia* [imported from Myanmar, as permitted by Korea Food and Drug Administration (KFDA)](approximately 2.8kg) was purchased from a market.

Methods

The air-dried root bark (2.8kg) of *P. corylifolia* were chopped and extracted with chloroform (5 L × 4) at room temperature for 7 day. The combined filtrate was concentrated in vacuo to yield a dark brown gum(386.4g). This crude extract (30.4g) was fractionated by silica gel flash CC employing a gradient of hexane to EtOAc.

In spectrophotometric experiments, enzyme activity was initial velocity (v_i) monitored by observing dopachrome formation at 475nm with a UV-vis spectrophotometer (Spectro UV-vis double beam; UVD-3500, Labomed, Inc.) at 30°C.

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All samples were first dissolved in EtOH at 10 mM. First, 66 μL of a 2.7 mM L-tyrosine (*K*m=180μM) aqueous solution was mixed with 824 μL of 0.25 M phosphate buffer (pH 6.8). Then, 100 μL of the sample solution and 10 μL of the same phosphate buffer solution containing mushroom tyrosinase (144 units) were added in this order to the mixture.

Results

In the preliminary screening, we observed that chloroform extracts of the seeds of *Psoraleacorylifolia* showed significant inhibition of L-tyrosinase oxidation. More detailed bioassay of the isolated compounds were subsequently conducted. All compounds (**1** - **5**) showed a dose-dependent inhibitory effect on monophenolase activity. As the concentrations of the inhibitors were increased, the residual enzyme activity drastically diminished (Fig. 1). The potency of compound **1**(IC_{50}=12.3 μM) can be favourably compared with commercially available inhibitor currently used as cosmetics, such as kojic acid (IC_{50}=16.2 μM). The potent inhibitors **1** - **4** exhibited the competitive inhibition characteristics in analysis of Lineweaver-Burks and Dixon-plot.

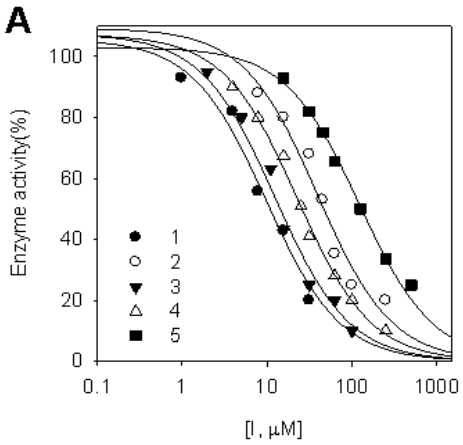

Fig1. Effect of compounds (**1**, **2**, **3**, **4**, and **5**) on the activity of tyrosinase for the catalysis of L-tyrosine.

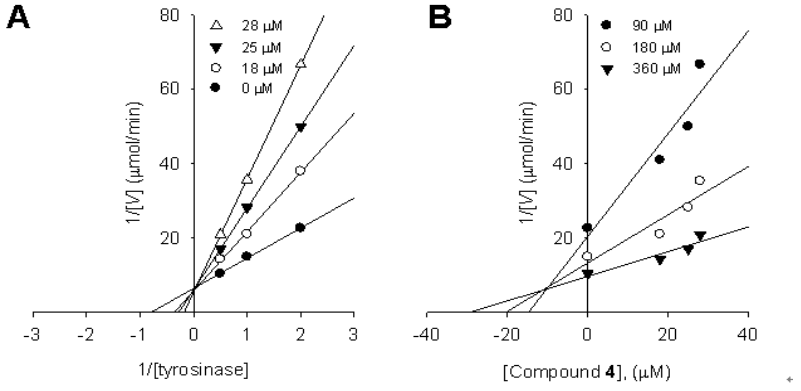

Fig2. Lineweaver-Burk and Dixon plots for the inhibition of compound **4** on the monophenolase activity of tyrosinase