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The evaluation of the synergistic effect of 3-(2,4-dihydroxyphenyl)propionic acid and L-ascorbic acid on tyrosinase inhibition

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Abstract: 3-(2,4-Dihydroxyphenyl)propionic acid (DDPA) and L-ascorbic acid (vitamin C) show tyrosinase inhibition activity. A synergistic effect on tyrosinase inhibition was observed when the two compounds were mixed. The effect significantly decreased the IC_{50} value of both compounds.

Keywords: 3-(2,4-dihydroxyphenyl)propionic acid (DDPA); L-ascorbic acid (vitamin C); synergistic effect; tyrosinase inhibition.

1 Introduction

Melanin pigmentation is an important factor which determines the colour of mammalian skin and hair. The synthesis of melanin needs tyrosinases for catalysis. Thus, the inhibition on tyrosinase is an effective method to avoid melanin pigmentation [1]. Tyrosinases (EC 1.14.18.1) are enzymes which have a binuclear copper centre in the catalytic centre. This catalytic centre is able to insert oxygen to an existing hydroxyl group in the ortho-position of an aromatic ring [2]. Furthermore, the subsequent oxidation of the diphenolic product will change into the corresponding quinone [3].

3-(2,4-Dihydroxyphenyl)propionic acid (DDPA) has a tyrosinase inhibition activity which has been characterised already [4, 5]. L-Ascorbic acid (vitamin C) is also widely used in cosmetics or food supplements for its antioxidant and tyrosinase inhibitor activities [6, 7]. In this study, we used mushroom tyrosinase to evaluate the synergistic effect of L-ascorbic acid and DDPA on tyrosinase inhibition.

Our findings indicated that DDPA's tyrosinase inhibition activity was stronger than L-ascorbic acid, as shown in Figure 1. IC_{50} values of 0.015 mg/mL and 0.96 mg/mL were estimated for DDPA and L-ascorbic acid, respectively. The IC_{50} value of DDPA was lower than that reported in the literature [8], but the value of L-ascorbic acid was similar to that reported in the literature [9].

The concentration of L-ascorbic acid was constant (0.5 mg/mL), but the activity increased after adding 0.0005 mg/mL of DDPA to the solution. The activity kept increasing continuously with the increase in DDPA concentration. Similarly, when the concentration of DDPA was kept constant (0.005 mg/mL), the activity increased after adding more L-ascorbic acid (Figure 1). In other words, the mixture of DDPA and L-ascorbic acid led to a decrease in the IC_{50} values. The IC_{50} value of DDPA decreased from 0.015 mg/mL to 0.0005 mg/mL (about 1/30) when 1.0 μ g of L-ascorbic acid (2 μ L of 0.5 mg/mL equivalent) was added to the DDPA solution. Similarly, the IC_{50} value of L-ascorbic acid decreased from 0.96 mg/mL to 0.06 mg/mL (about 1/16) when 10 ng of DDPA (2 μ L of 0.005 mg/mL equivalent) was added to the L-ascorbic acid solution, as shown in Table 1.

From the results of this research, we can conclude that the mixture of DDPA and L-ascorbic acid leads to have a significant increase in tyrosinase inhibition activity and that both compounds show a synergistic effect on each other's activity.

Since L-ascorbic acid has been found to have several functions widely applied in cosmetics and medicines, it is recommended that further research be carried out also on its synergistic effect with DDPA in the area of cosmetics and medicines.

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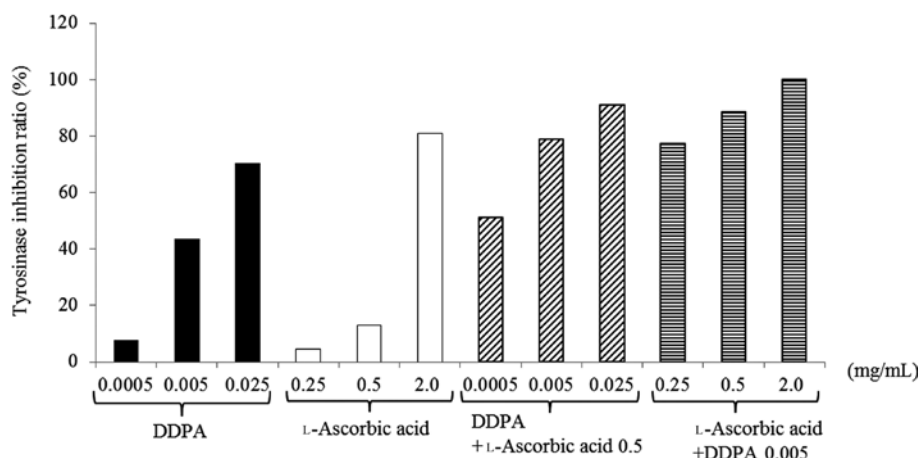


Figure 1: The IC_{50} of 3-(2,4-dihydroxyphenyl)propionic acid (DDPA), L-ascorbic acid and the mixture of two compounds at different concentrations.

Table 1: IC_{50} of 3-(2,4-dihydroxyphenyl)propionic acid (DDPA) and L-ascorbic acid for tyrosinase inhibition.

IC_{50} (mg/mL)	DDPA	L-Ascorbic acid	DDPA+L-ascorbic acid	
			DDPA	L-Ascorbic acid
Concentration	0.015	0.96	0.0005	0.06

2 Experimental

DDPA was purchased from Fluka (Japan, purity $\geq 95.0\%$). L-Ascorbic acid was purchased from Wako Pure Chemical Industries (Japan, purity 99.6%). Dimethyl sulfoxide (DMSO) was purchased from JUNSEI (Japan). Tyrosinase from mushroom (purity ≥ 1000 unit/mg), 3,4-dihydroxy-L-phenylalanine (L-DOPA, purity 98%), both were obtained from Sigma (St. Louis, MO, USA). Potassium dihydrogen phosphate and di-potassium hydrogen phosphate were obtained from Wako Pure Chemical Industries Ltd (Japan). The xMark™ Microplate Absorbance Spectrophotometer was obtained from Bio-Rad (USA).

The stock solutions of DDPA and L-ascorbic acid were prepared by dissolving their reference standards in DMSO and the final concentrations was 1.0 mg/mL, respectively. All solutions were protected from light and stored at 4 °C.

For the tyrosinase inhibition activity experiment, the samples' solutions were diluted in DMSO to form a series of concentrations. Tyrosinase inhibitory activity was determined by a spectrophotometric method which was described by Chan et al. [10] using a modified dopachrome method with L-DOPA as the substrate. The reaction mixture contained phosphate buffer (70 μ L, 0.1 M, pH 6.8), inhibitor (2 μ L) dissolved in DMSO and

tyrosinase aqueous solution (30 μ L, 71.5 units/mL). The reference solution was prepared with 2 μ L of DMSO instead of inhibitor. After adding the tyrosinase solution, the reaction mixture was incubated at 25 °C for 5 min. After incubation, half of both samples with inhibitor and reference samples were selected to be blank samples and reference blank samples, which were added by 30 μ L of water instead of 30 μ L of 10 mM L-DOPA. Thirty microlitres of 10 mM L-DOPA was added to the rest of samples and the 96-well microplate was incubated at 25 °C for 2 min. The absorbance was measured at 475 nm. Results were compared with a positive control and a blank containing DMSO in place of the sample solution and H₂O in place of L-DOPA. The percentage of tyrosinase inhibition was calculated as follows:

$$\text{Tyrosinase inhibition (\%)} = \left(1 - \frac{A_{\text{Sample}} - A_{\text{blank}}}{A_{\text{Ref.}} - A_{\text{Ref. blank}}} \right) \times 100$$

($n=4$, SD=10).

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