

TYROSINASE INHIBITORY ACTIVITY OF *LONICERA JAPONICA*

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A tyrosinase inhibitor, Lonjapoin, was isolated from the ethanolic extracts of Flos *Lonicera* by activity-guided fractionation. Lonjapoin (IC₅₀; 2.07 µg/mL) was found to be potentially as effective inhibitor of production of melamin.

Flos *Lonicera* (Jinyinhua) is the dry flower buds of *Lonicera japonica*. Thunb. It is used as an antibacterial and antiphlogistic agent in the treatment of abscess, laryngeal catarrh, erysipelas, dysentery, cold and fever. In our continuing search for natural source as cosmetic ingredients from Chinese medicinal herbs and found the extracts of Flos *Lonicera* showed the significant tyrosinase inhibition activity. In this study the active constituent of *Lonicera japonica*. obtained with the process of tyrosinase activity assay and column chromatography.

EXPERIMENTS

Extraction and Isolation

The flos *Lonicera* (350 g) was cut in pieces and extracted by refluxing with 50% ethanol (3 L) for 3 h and then filtered. The filtrate was evaporated to dryness *in vacuo*. The extract was dissolved in water and partitioned with ethyl acetate and n-butanol three times, respectively. After remove the solvent *in vacuo*, the ethyl acetate soluble fraction, n-butanol soluble fraction and aqueous soluble fraction were examined

with tyrosinase inhibitory activity assay. The n-butanol soluble fraction was column chromatographed on Diaion LH-20 and obtained an active constituent, Lonjapoin.

Assay of Tyrosinase Inhibitory Activity

Tyrosinase inhibitory activity was assayed by the dopachrome method with slight modification¹. One hundred mL of mushroom tyrosinase solution (350 U/mL), with or without 1 mL of L-tyrosine (0.03%, w/v), 0.9 mL of Sorensen's buffer (pH 6.8) and 1 mL of solvent with or without the test specimen, were mixed in the test tube, incubated at 37°C for 25 min, and the absorbance of each tube measured at 475 nm with a Shimadzu UV-260 spectrometer after incubation. The percentage inhibition of tyrosinase was calculated as follows :

$$\text{tyrosinase inhibition (\%)} = [(A_b - A_0) - (A_t - A_1)] / (A_b - A_0) \times 100$$

A_t : the absorbance in the presence of the L-tyrosinase and test specimen after incubation

A_1 : the absorbance in the presence of the test specimen and without L-tyrosinase after incubation

A_b : the absorbance in the presence of the L-tyrosinase and without test specimen after incubation

A_0 : the absorbance in the absence of the test specimen and L-tyrosinase after incubation

Result and Discussion

We examined the tyrosinase inhibitor isolated from the Flos Lonicera by activity-guided fractionation. In this study, Lonjapoin was identified as a new inhibitor of mushroom tyrosinase. This is the first report of antityrosinase activity of Flos Lonicera.

Table I. Tyrosinase inhibitory activity of the Flos Lonicera and Lonjapoin

	Concentration (mg/mL)	inhibition (%)
50% ethanol ext.	4	24.7
	2	22.8
	1	20.7
n-butanol lay.	4	29.5
	2	23.2
	1	20.7
n-butanol lay. (H ₂ O/CH ₃ OH)=1/1 frac.	4	35.9
	2	35.6
	1	34.6
.Lonjapoin*	4	55.0
	2	53.4
	1	40.0

* IC₅₀ (mg/mL)=2.07

References

1. V. J. Hearing, Jr., "Methods in Enzymology", vol. 142, ed. by S. Kaufman Academic Press, New York, 1987, p 154..