



ฤทธิ์ของสารต้านอนุมูลอิสระและหยุดยั้งเอนไซม์ไทโรซิเนสของรังไหมและใบหม่อน

## Tyrosinase Inhibitory and Antioxidant Activities of Silk Cocoons and Mulberry Leaves

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**บทคัดย่อ:** รังไหมและใบหม่อนได้ถูกนำมาใช้ทางด้านการแพทย์ เครื่องสำอางและอาหาร จุดประสงค์ของการศึกษานี้ ได้แก่การทดสอบฤทธิ์ของสารต้านอนุมูลอิสระจากรังไหมและใบหม่อน โดยใช้ 1,1-diphenyl-2-picryl-hydrazyl radical และ thin-layer chromatography (TLC) และทดสอบฤทธิ์ในการหยุดยั้ง tyrosinase โดยใช้ dihydroxyphenylalanine พบว่าสารสกัดด้วยน้ำและแอลกอฮอล์จากรังไหม 3 ชนิด (นางน้อย UB1 และลาว) และใบหม่อนมีฤทธิ์ของสารต้านอนุมูลอิสระและหยุดยั้ง tyrosinase อย่างไรก็ตามสารสกัดจากทุกตัวอย่าง (1,000 µg/reaction mixture) หยุดยั้ง tyrosinase 12.28-45.98% ซึ่งต่ำกว่าสารมาตรฐาน kojic acid มาก ( $IC_{50} = 0.45$  µg/reaction mixture) ผลจาก TLC แสดงว่าสารสกัดด้วยแอลกอฮอล์จากรังไหมทั้ง 3 ชนิดมี flavonoids แต่สารสกัดจากนางน้อยเท่านั้นที่มี carotenoid นอกจากนี้การแยกสารต่างๆ ออกจากกันทำลายสารที่มีฤทธิ์ต้านอนุมูลอิสระสูง ดังนั้น การแยกสารออกจากกันจะมีข้อเสียที่ทำให้ค่าใช้จ่ายเพิ่มขึ้นและฤทธิ์ของสารต้านอนุมูลอิสระลดลง

**Abstract:** Silk cocoons and mulberry leaves have been used in the field of medicines, cosmetics, and foods. The objective of this study is to determine the antioxidant activities of silk cocoons and mulberry leaves using 1,1-diphenyl-2-picryl-hydrazyl radical and thin-layer chromatography (TLC), and to determine tyrosinase inhibitory activities using dihydroxyphenylalanine. The water and ethanol extracts from silk cocoons (Nang Noi, UB1, and Lao) and mulberry leaves showed antioxidants and tyrosinase inhibitory activities. However, the extracts from all samples at 1,000 µg/reaction mixture inhibited tyrosinase in the range of 12.28-45.98%, which was much lower than the standard whitening agent kojic acid ( $IC_{50} = 0.45$  µg/reaction mixture). The results from TLC showed that the ethanol extracts from the 3 species of cocoons contained flavonoids, but only the extract from Nang Noi contained carotenoid. In addition, the separation destroyed the fraction with high antioxidant activity. Therefore, the disadvantage of the extract separation is increased cost and decreased antioxidant activities.

**Introduction:** Tyrosinase inhibitors have been used in cosmetics as whitening agent and antioxidants have been used in drugs, cosmetics, and foods to eliminate free radicals and reactive oxygen species the cause of several diseases and aging. Kato et al reported that the water extract from a species of silk cocoons had antioxidant and tyrosinase inhibitory activities<sup>1</sup>. The objective of this study is to determine antioxidant and tyrosinase inhibitory activities of the water and ethanol extracts from mulberry leaves and 3 species of silk cocoons.

**Methodology:** Three species of silk cocoons *Bombyx mori* (Nang Noi, yellow cocoons; UB1, white cocoons; and Lao, cream cocoons) and mulberry (*Morus alba* L.) leaves were

kindly supplied by Radiation Entomology Group of Office of Atoms for Peace and Institute of Sericulture. The cocoons were cut into pieces about 1 centimeter square. For the water extracts, they were placed in 90°C distilled water (water bath) for 2 hours; for the ethanol extracts, they were placed in ethanol at room temperature overnight. For the water and ethanol extracts of mulberry leaves, they were blended with water or alcohol at room temperature. The extracts were filtrated and dried by lyophilization. The amount of protein in water extracts from cocoons was determined using Coomassie Brilliant Blue G-250 (spectrophotometric)<sup>2</sup>. Antioxidant activities were determined using 1,1-diphenyl-2-picrylhydrazyl radical (spectrophotometric)<sup>3</sup> and thin-layer chromatography (TLC), and tyrosinase inhibitory activities were determined using dihydroxyphenylalanine as a substrate<sup>4</sup>. Ascorbic acid was used as a standard for antioxidant and kojic acid was used for tyrosinase inhibitors.

### Results, Discussion and Conclusion:

The water and ethanol extracts from mulberry leaves and 3 species of silk cocoons showed antioxidant activities (Table 1) and tyrosinase inhibitory activities (Table 2). For antioxidant activities, the inhibitory concentration<sub>50</sub>; IC<sub>50</sub> of all samples (185-659 µg/reaction mixture) was about one order lower than IC<sub>50</sub> of ascorbic acid (29.5 µg/reaction mixture) (Table 1). For tyrosinase inhibitory activities, the extracts from all samples at 1,000 µg/reaction mixture inhibited tyrosinase in the range of 12.28 - 45.98% (Table 2), which is similar to the results reported by Kato et al. (the 1,000 µg of water extract from 1 type of silk cocoon exhibited tyrosinase inhibitory activities 24%). However, this inhibitory activities was very low compared to the standard whitening agent kojic acid (Figure 1; IC<sub>50</sub> = 0.45 µg/reaction mixture).

The results from TLC showed that the ethanol extracts from the 3 species of cocoons contained flavonoids, but only the extract from Nang Noi contained carotenoid. Therefore, antioxidants in silk cocoons are not only from water soluble protein sericin, but also from flavonoids and carotenoid in ethanol extracts. In addition, the results from TLC showed that the fraction with high antioxidant activity lost its activity after separation. Therefore the separation of the extract not only increases cost, but also decreases antioxidant activity.

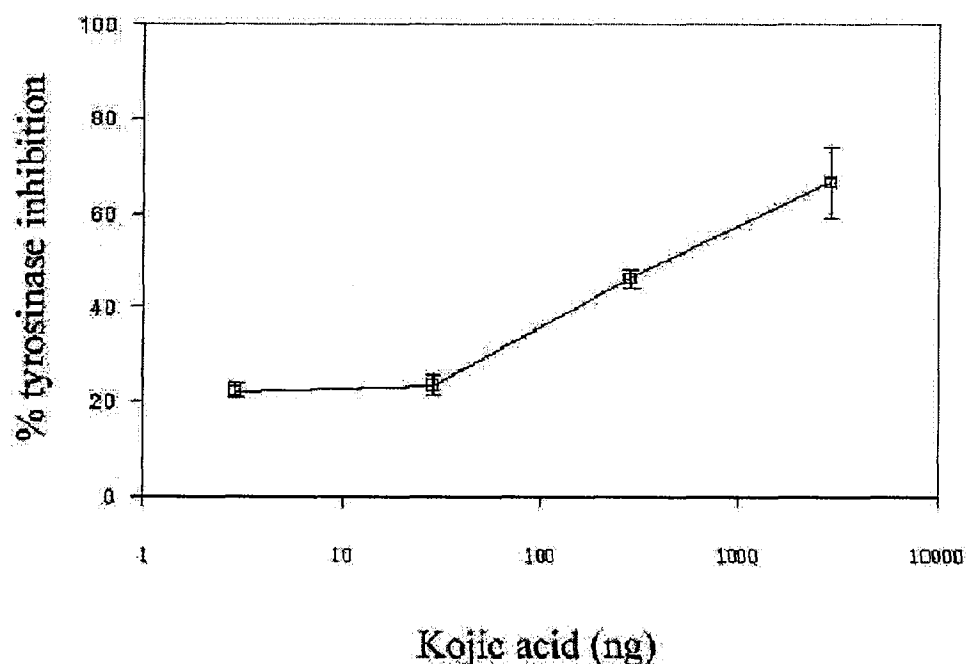
Table 1 DPPH inhibition

Samples	IC <sub>50</sub> (µg/reaction mixture)	
	Water extracts	Ethanol extract
Cocoons		
Nang Noi	292	185
UB 1	651	580
Lao	463	222
Mulberry leaves	659	551

Note: Ascorbic acid exhibited IC<sub>50</sub> at 29.5 µg/reaction mixture.

**Table 2** Percentage of tyrosinase inhibition using the extracts 1,000 µg/reaction mixture

Samples	% tyrosinase inhibition	
	Water extracts	Ethanol extract
Cocoons		
Nang Noi	45.98±3.0	21.11±1.1
UB 1	22.67±2.1	18.24±1.0
Lao	28.66±1.4	15.46±1.9
Mulberry leaves	12.28±1.2	16.78±1.5



**Figure 1** Percentage of tyrosinase inhibition by kojic acid

**References:**

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**Keywords:** antioxidant, tyrosinase inhibitor, whitening agent, silk cocoon, mulberry leaves