CHAPTER IV

APPLICATION OF SILKWORM EXCRETA EXTRACT

4.1 Introduction

It is known in Far East countries that silkworm excreta have been used as traditional medicine for a long time. They also have a benefit for agricultural purposes. Nowadays, there are new attempts to use the extract from silkworm excreta as natural colorant. The mulberry leaves which are the feed for silkworm have been reported for a number of bioactive chemical constituents. So, it is interesting that whether the silkworm excreta contain these beneficial compounds and possessed the same bioactivity or not.

Thus, in this chapter, bioactivity of silkworm excreta extracts was observed including free radical scavenging and tyrosinase inhibitory activity. Because the activities would guide the application of silkworm excreta extract as a whitening ingredient in cosmetic. Moreover, the stability of the silkworm excreta extract was studied as well.

4.2 Material and methods

4.2.1 Bioactivity test of crude silkworm excreta extract

The silkworm excreta extracts obtained by using four selected solvent including hexane, acetone, ethanol, and hot water (as described in 3.2.3) were evaluated for their free radical scavenging and tyrosinase inhibitory activity.

4.2.1.1 Free radical scavenging activity of the crude extracts

Each crude extract was diluted with methanol and was tested for free radical scavenging activity by DPPH assay as described in 3.2.6.

4.2.1.2 Tyrosinase inhibitory activity of the crude extracts

Each crude extract was diluted with methanol and was tested for Tyrosinase inhibitory activity by DPPH assay as described in 3.2.6.2.

4.2.2 Application of silkworm excreta extract in cosmetic

Compatibility of crude silkworm excreta extract obtained by using acetone as an extracting solvent with the selected cosmetic model was observed due to the acceptable color and the strong free radical scavenging activity. The Cold cream USP XXI was selected as a cosmetic model. Cold cream USP XXI is a water in oil (W/O) emulsion which has been used as a cream base for various skin preparations. Cold cream are generally not rinsible, are considered greasy and inelegant and are tissued off the skin, they leave behind a film that has proven moisturizing characteristics (Schmitt, 1996).The high oil content makes the formulation favorably compatible with the compounds that has low polarity in silkworm excreta extract.

Cold cream USP XXI

Cetyl ester wax	125	g
White wax	20	g
Mineral oil	560	g
Sodium Borate	5	g
Purified Water	190	mL

Acetone extract (0.05%) was used as colorant for cold cream by two preparation methods. Firstly, it was mixed with other ingredients in the emulsion preparation process. The oil phase was heated at 70^{o}C until waxes completely melted, at the same time water phase was also heated at 70^{o}C in different container. Then, two phases were mixed and stirred until the emulsion congeals. Another method to color the cold cream is the direct addition of the extract into the finished cream (cold mixing).

4.2.3 Heat stability of crude silkworm excreta extract

When making of the emulsion in cosmetic, heat is employed to dissolve waxes (oil phase) before mixing with aqueous phase, and then the colorant inevitably expose to the high temperature until the mixture congeals. In finished products, stability testing is done to ensure that a developed product will be fit for use during its expected life (Schmitt, 1996). Thus, crude acetone extract was subjected to the stability testing in solution form as well as in cosmetic preparation.

4.2.3.1 Heat stability of the crude extract in solution

This experiment was done to observe whether the activity of the extract affected by heat exposure or not. Because heat is generally employed in preparation of cosmetic emulsion and might have an effects on bioactivity of the ingredient. The crude acetone extract was dissolved in ethanol and heated at 70^{o}C for 2 h. Samples were collected every 15 min to check the free radical scavenging activity by DPPH assay and tyrosinase inhibitory activity.

4.2.3.2 Heat stability of the crude extract in cosmetic model

Silkworm excreta extract have been discovered for the use as colorant. However, no published data reported on the stability of silkworm colored extract in the cosmetic model under stress condition.

The stress condition was applied to accelerate the degradation of components which are responsible for the color of silkworm excreta extract. The suggested stress condition was at least 6 or 8 heating/cooling cycle between refrigerator temperature and 45^{o}C with storage of each temperature of no less than 48 h (Idson, 1998). In this experiment, the colored cold cream prepared by cold mixing method was kept in a tight container and underwent through the heating/cooling cycle which was 48 h in 4^{o}C followed with 48 h in 45^{o}C for 6 cycles. Color variation at the end

of the experiment was observed as well as change of cream base texture and appearance.

4.3 Results and discussion

4.3.1 Bioactivity of silkworm excreta extracts

4.3.1.1 Free radical scavenging activity of the crude extracts

Due to the previous reports on biological activity of mulberry leaves extract, free radical scavenging activity and tyrosinase inhibitory activity were selected to examine the activity of the silkworm excreta extracts.

As shown in Figure 6, crude acetone extract obtained by using acetone as extracting solvent exhibited high free radical scavenging activity when compared with positive control, quercetin (91 and 89 percent at 800 μg/ml, respectively). In addition, crude extracts obtained by hot water and ethanol also exhibited high free radical scavenging activity (more than 50%) at 800 μg/ml dose.

Although ethanol, methanol, and aqueous alcohol have been extensively reported as suitable solvents for extraction of antioxidant such as phenolic compounds and flavonoids from plant sample (Khodaparast *et al.*, 2007; Ning *et.al.*, 2008; Stanojevic *et al.*, 2009), acetone was more favorable in extraction of the antioxidants from silkworm excreta when compare to other solvents. It implied that there are some other compounds besides phenolic compounds and flavonoids that have free radical scavenging activity could be extracted by this solvent.

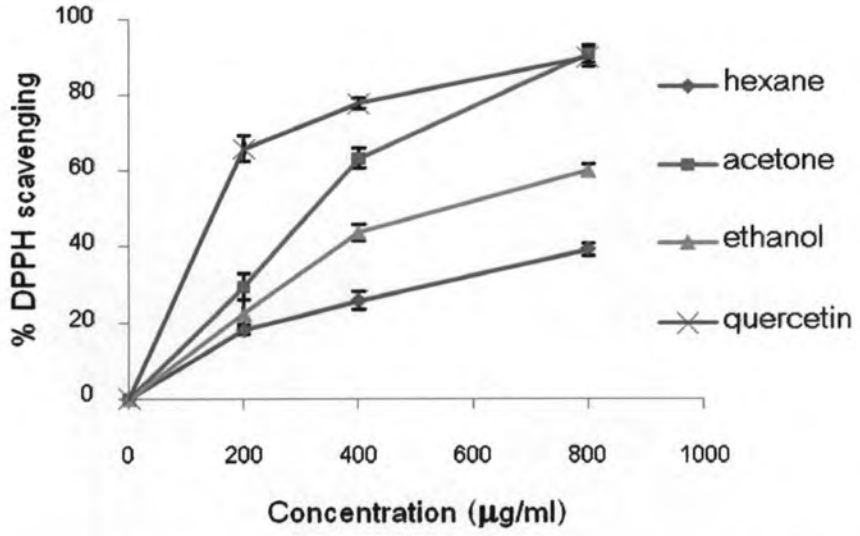

Figure 6 DPPH scavenging activity of crude silkworm excreta extracts. Each value is presented as mean ± S.D. (n=3). The vertical bars represent standard deviation.

4.3.1.2 Tyrosinase inhibitory activity of the crude extracts

The results of mushroom tyrosinase inhibition activity of crude extracts were illustrated in Figure 7. All of the crude extracts from silkworm excreta had low activity against mushroom tyrosinase when compared with standard reference, kojic acid. None of the extracts showed more than 50% inhibition even though at the highest concentration used in the experiment (800 μg/ml). The result indicates that the potent tyrosinase inhibitors such as oxyresveratrol (Shin *et al.*, 1998) and mulberroside F (Lee *et al.*, 2002) found in mulberry leaves have already been transformed to other inactive compound during the digestion process of silkworm or have been excreted by other route. Thus, for this experiment, silkworm excreta extract is not suitable for using as a whitening ingredient due to the low activity in mushroom tyrosinase inhibition.

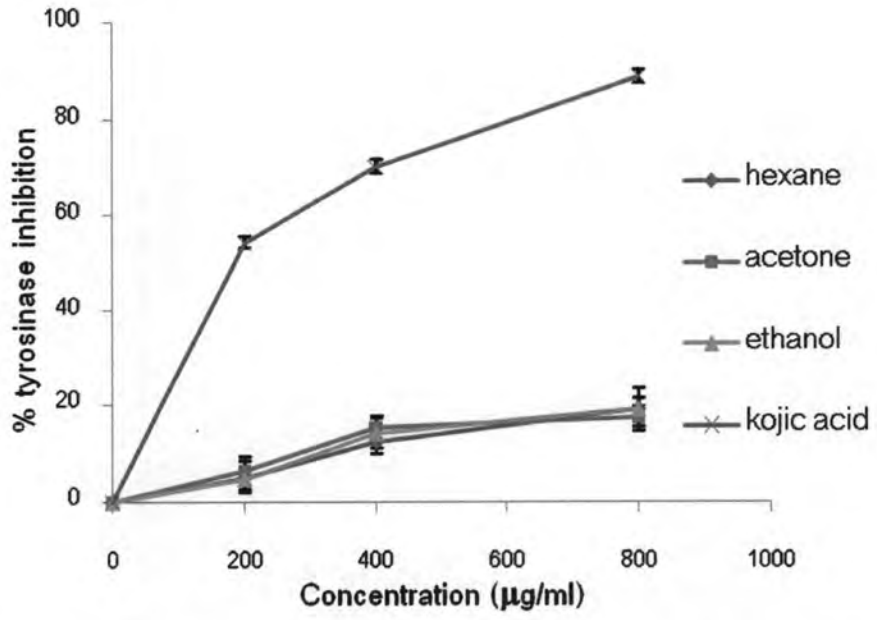

Figure 7 Tyrosinase inhibitory activity of crude silkworm excreta extracts. Each value is presented as mean ± S.D. (n=3). The vertical bars represent standard deviation.

4.3.2 Application of silkworm excreta extract in cosmetic

Silkworm excreta extract obtained by using acetone as an extraction solvent was evaluate for its compatibility to the cosmetic model, Cold cream USP XXI. Acetone extract was applicable for Cold cream USP XXI as a colorant in both preparative methods. No physical deterioration such as phase separation observed in finished product.

4.3.3 Heat stability of crude silkworm excreta extract

4.3.3.1 Heat stability of the crude extract in solution

As the previous result about bioactivity of silkworm excreta extract, acetone extract showed high free radical scavenging activity and heat exposure may affect the bioactivity of the extract.

Figure 8 demonstrated the changes in activity of the acetone extract in the term of percentage of DPPH reduction and tyrosinase inhibition. When expose to the heat at 70°C for 2 h, the DPPH reduction activity was decreased as the time passed by. The decrease of activity was high after 30 min indicates that the compounds responsible

for the radical scavenging activity were changes into the inactive form or degraded through the reaction affected by high temperature. Thus, in the preparation of cosmetic, silkworm excreta extract should not be exposed to the heat longer than 30 min. Regarding to tyrosinase inhibitory activity, the changes in % inhibition was very few.

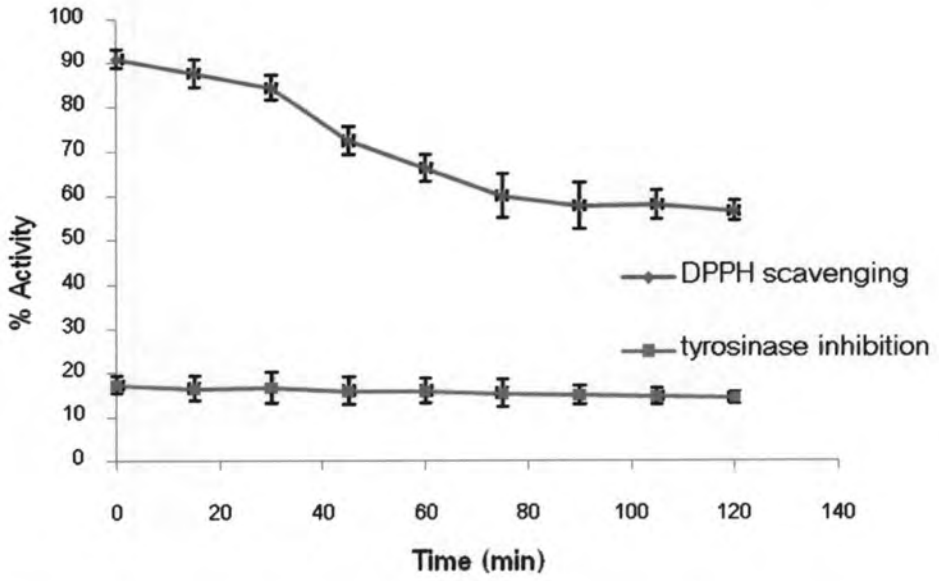

Figure 8 % DPPH reduction and tyrosinase inhibition of crude acetone extract (800mg/ml) during heat treatment. Each value is presented as mean ± S.D. (n=3). The vertical bars represent standard deviation.

4.3.3.2 Heat stability of the crude extract in cosmetic model

About the cream texture, no visible difference was detected after finish 6 heating/cooling cycles. Regarding the color of cold cream, color was changed to paler green (Figure 9). The result indicates that the pigments which are responsible for green color of the extract such as chlorophylls and xanthophylls were degraded under stress condition (Nakatani *et al.*, 1981).

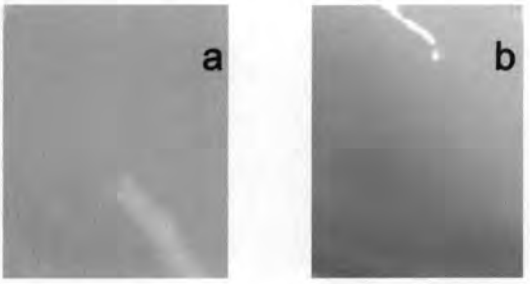

Figure 9 Cold cream colored by crude acetone extract. a) before and b) after 6 of freeze-thaw cycles.

4.4 Conclusion

Crude silkworm excreta extract obtained by maceration with acetone and accelerate the extraction by sonication exhibited high activity against DPPH but weak against mushroom tyrosinase. Crude acetone extract was applicable for Cold cream USP XXI as a colorant in both preparative methods. Therefore, the extract could be applied as a colorant for Cold cream USPXXI.

The stability to heat of the silkworm excreta extract obtained by using acetone as an extracting solvent in solution was observed. Heat treatment at 70°C induced degradation of compound that responsible for the free radical scavenging activity. The decrease of the free radical scavenging activity was rapid after 30 min, thus longer exposure to the heat should be avoided. Regarding the stability of finished product under stress condition, no visible changes in the texture was detected. The color of product changed to paler green.