



## Optimizing mulberry leaves extract conditions for the preparation in the lotion formulation

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### Introduction

Herbs and herbal products have gained its momentum as alternative choices as food supplement due to an abundance of biologically active compounds<sup>1</sup>. Many Thai herbal plants are major sources of phenolic compounds, which are synthesized as secondary metabolites during general growth and development and in responses to environmental stimuli such as UV radiation and wounding by insects or microbes<sup>2</sup>.

Mulberry (*Morus alba* L.) is a widely distributed deciduous tree belonging to the Moraceae family. It is widely cultivated in various parts of Thailand for food and beverage industry. Various phenolic compounds in mulberry fruits including quercetin, kaempferol and rutin have been found to promote strong antioxidant activity. Mulberry leaves contain diverse chemical compounds such as flavonoids, polysaccharides, alkaloids, sterols, volatile oil, amino acids, vitamins and other trace elements.<sup>3,4</sup> Flavonoids in mulberry leaf, including of flavonol, flavones, and anthocyanidin are present in high amount. Many studies suggest that enhance cerebrovascular and coronary blood flows, regulate arrhythmia, soften angiosclerosis, as well as decrease sugar and fat (references). Flavonoids from the leaf extracts has also been found to exhibit antioxidant and anticancer activity<sup>4</sup>. However, significant differences in the flavonoid content can be observed in diverse organs, habitats, periods and varieties<sup>5</sup>.

To the best of information on the extraction condition and its effect on bioactivities of mulberry leaves is not available. Therefore, this study aims to examine the optimum condition to extract mulberry leaves that will yield the maximum antioxidant activity and to formulate lotion product with mulberry leaf extract.

### Methods

#### *Plant material*

Fresh mulberry leaves were collected in June to October 2016 from Sisaket province. The Plant samples were cleaned, dried in an oven air at 30°C for 6 h, ground and passed through 20 mesh sieve.

#### *Preparation of plant extract*

Powdered plants were divided into four groups and separately extracted to observe an effect of each variable to the antioxidant activity. The extraction procedure is as follows:

- 1) To determine the optimum ethanol concentration, one gram of mulberry leaf powder was extracted by refluxing with 40 mL of ethanol (30%, 45%, 60%, 75%, and 90%) for 30 minutes at 85 °C.
- 2) To determine the optimum extraction time, one gram of mulberry leaf powder was separately extracted by refluxing with 40 mL of 45% (v/v) ethanol for different time (30 min, 60 min, 90 min, 120 min and 150 min) at 85 °C.
- 3) To determine the optimum liquid-to-solid ratio, one gram of mulberry leaf powder from Sisaket province was extracted by refluxing with different volume (10 mL, 20 mL, 30 mL, 40 mL and 50 mL) of 45 % for 30 minutes at 85 °C.
- 4) The effect of temperature, one gram of mulberry leaf powder from Sisaket province was extracted by refluxing with 40 mL of 45 % ethanol for 30 minutes at different temperature (75 °C, 80 °C, 85 °C, 90 °C and 95 °C).

All of extract solutions were dried using rotary evaporator and stored at 4°C for further studies. The stock solution of each extract was prepared at the concentration of 10 mg/mL.

#### *Determination of antioxidant activity by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging (DPPH) assay*

## 1) Preparation of standard

Ten milligrams of ascorbic acid was accurately weighed in 10 ml volumetric flask and adjusted with distilled water to be used as primary standard solution. The secondary standard solution was obtained by diluting primary standard solution to the final concentration of 0.1 mg/ml. The working solution at the concentrations of 2-15 ppm were prepared and used to construct a calibration curve of ascorbic acid.

## 2) Preparation of sample

All extracts were weighed in 10 ml volumetric flask and adjusted with different concentration of ethanol to obtained 0.5 mg/mL of working solution.

3) Determination of antioxidant activity<sup>6,7</sup>

One hundred microliters of each working standard and sample solution was mixed with one hundred microliters of 208  $\mu$ M DPPH in ethanol. After a dark treatment for 30 minutes, the absorption of each solution was measured at 515 nm using a microplate reader. The %inhibition of sample was calculated using the ascorbic acid standard curve. The results were expressed as mean  $\pm$  standard deviation (SD).

$$\% \text{ inhibition} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100$$

Where  $A_{\text{control}}$  = Absorbance of control  
 $A_{\text{sample}}$  = Absorbance of sample

**Lotion formulation**

EDTA, glycerin and xanthan gum were weighed and dissolve with water. Mulberry leaf extract (0.05% w/w) was first dissolved in a little amount of water, then added to the mixture. Other ingredients, including cetyl alcohol, GMS, stearic acid, mineral oil, isopropyl myristate, dimethicone, vitamin E and novomer, were separately weighed. The two parts of mixture were combined and mixed by a homogenizer. The finished lotion product was examined for physicochemical properties (pH, color, odor and precipitate) at  $4 \pm 2^\circ\text{C}$  and room temperature.

**Results****Determination of antioxidant activity by DPPH assay**

The concentration of ethanol that is used for the extraction can influence the antioxidant activity. The effect of ethanol concentration on antioxidant activity is shown in Figure 1A. The %inhibition increased from  $55.39 \pm 0.36$  to  $64.19 \pm 0.41$  when the concentration of ethanol increased from 30% to 75%. As the concentration of ethanol increase to 90%, the %inhibition decreased to  $52.95 \pm 0.00$ .

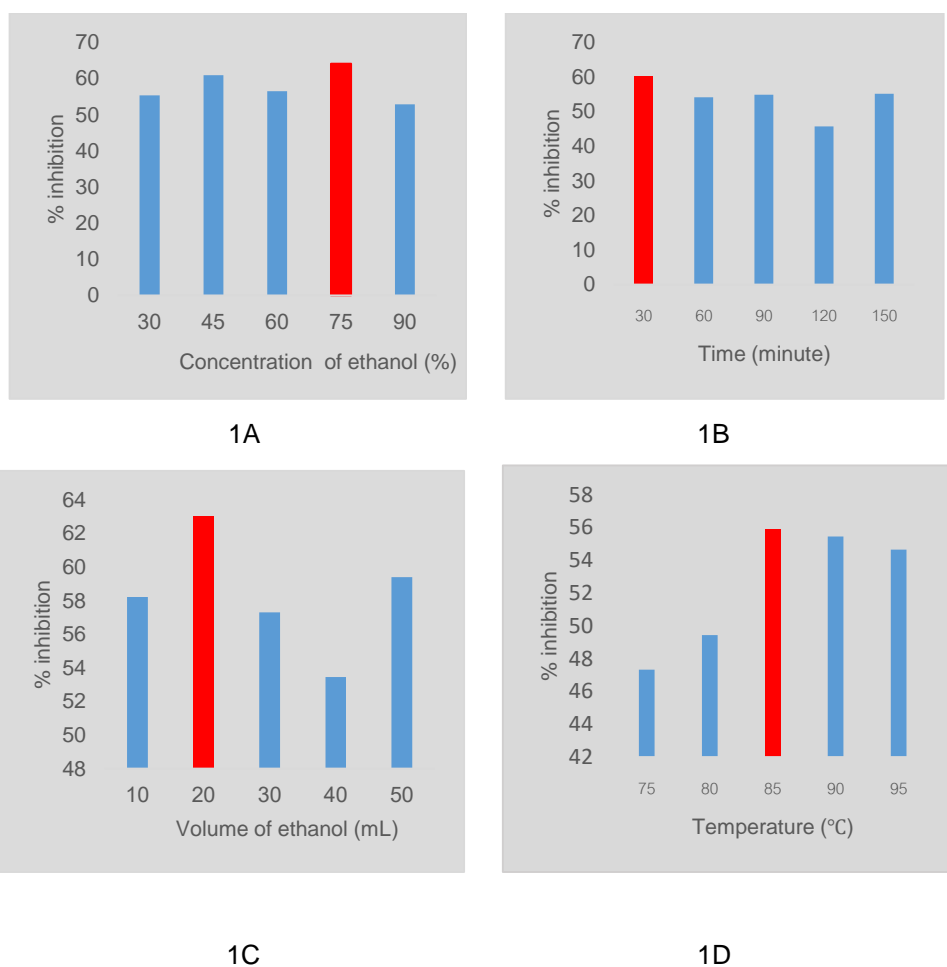
The effect of different times of extraction on the antioxidant activity is shown in Figure 1B. The optimum extraction time was 30 min as it gave the highest antioxidant activity. When extraction time was decreased to 60 and 90 min, the antioxidant activity decreased. The antioxidant activity was the lowest at 120 min.

The antioxidant activity was highest when the liquid-to-solid ratio was 1:20 w/v (Figure 1C). The effect of extraction temperature on antioxidant activity is shown in Figure 1D. The optimum temperature that yielded the highest antioxidant activity was  $85^\circ\text{C}$  while  $70^\circ\text{C}$  gave the lowest antioxidant activity.

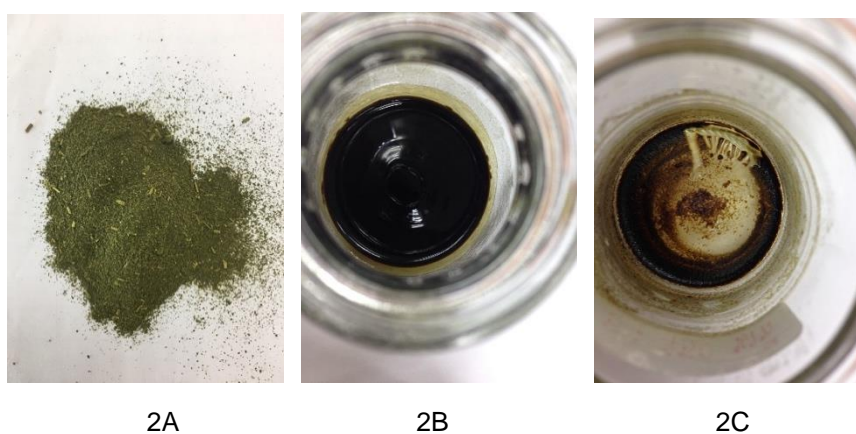
From the four previous experiments, the optimal extraction conditions were 75% (v/v) ethanol, 30 min extraction time and 1:20 (w/v) solid-to-liquid ratio and  $85^\circ\text{C}$  extraction temperature. Ground mulberry leaves were extracted under these conditions to quantify the antioxidant activity. The antioxidant activity value ( $\text{IC}_{50}$ ) from the optimum condition was  $0.890 \pm 0.15$  mg/mL compared with ascorbic acid (Table 1). The extract obtained from the optimal condition was greenish black in color and had a slight mulberry odor (Figure 2C)

**Table 1** The antioxidant activity value of Ascorbic acid and Mulberry leaf extract

Sample	$\text{IC}_{50}$	Linear equation	$R^2$
Ascorbic acid	0.0116 mg/mL	$y = 3.653x + 7.3137$	0.99321
Mulberry leaf extract	0.890 mg/mL	$y = 0.0521x + 3.5867$	0.99402



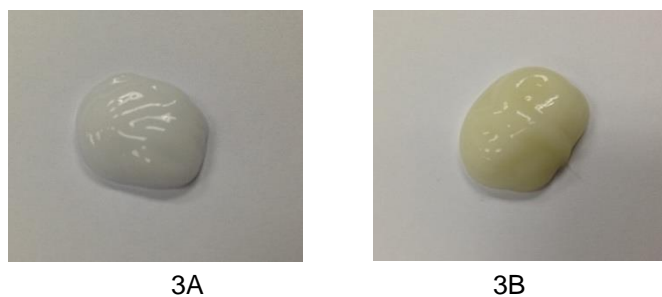
**Figure 1** The effects of extraction parameters on ascorbic acid equivalent antioxidant capacity. (A) Effect of ethanol concentration (B) Effect of extraction time (C) Effect of liquid-to-solid ratio and (D) Effect of extraction temperature



**Figure 2** The physical property of mulberry leaf (A) mulberry powdered (B) mulberry extract (C) mulberry dried extract

#### **Lotion formulation**

The based formulation and the mulberry leaf extract formulation for the lotion were obtained and physicochemical properties were analyzed. It was found that both formulations had a pH of 6.00. The mulberry leaf extraction formulation was light green in color with a slight mulberry odor. The based formulation was white (Figure 2) with slightly waxy odor. They did not precipitate when kept at 4 or 25 °C.



**Figure 3** Texture of the finished lotion (A) based formulation and (B) mulberry leaf extract formulation

**Table 2** Physicochemical properties of based formulation and Mulberry leaf extract formulation

Sample	pH		color		odor		precipitation	
	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C	4 °C	25 °C
<b>Based formulation</b>	6	6	white	white	Slightly wax	Slightly wax	No	No
<b>Mulberry leaf extract formulation</b>	6	6	Light green	Light green	Slightly mulberry	Slightly mulberry	No	No

## Discussion

A biological activity of herbal extracts is determined by on a number of factors such as time of harvest, growing condition and location, and extraction methods.<sup>8</sup> Depending on the target activity, extraction conditions may need to be tailored to optimize the yield of compounds of interest. In this study, it was shown that different conditions could influence the antioxidant activity of the mulberry leaf extract. A 75% ethanolic extract was the optimal ethanol concentration that yielded maximum antioxidant activity. This could be due to an abundance of less polar antioxidant compounds that were more readily extracted in the presence of ethanol, which is less polar than water. The extraction time and temperature were also factors that were included in this study as these two factors could significantly affect the yield and biological activities. These two factors may interact as an extraction at lower temperature may require a longer extraction time and vice versa. For some compounds that are heat-tolerant and are not easily degraded at high temperature, extraction at higher temperature and shorter time may be more efficient as demonstrated by this study. This suggests that antioxidant compounds in mulberry leaves may be less polar and not easily degraded by heat.

## Conclusion

This study demonstrated that the optimal extraction conditions for mulberry leaves were 75% (v/v) ethanol, 30 min extraction time, 1:20 (w/v) solid-to-liquid ratio and 85 °C extraction temperature. The extract obtained from these conditions exhibit IC<sub>50</sub> value of 0.890±0.15 mg/mL. The extract could be used in a lotion preparation as an additive antioxidant ingredient in a natural cosmetic skin care.

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