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Research Article

In Vivo Study on the Evaluation of a Sleeping Mask Gel Containing Red Cotton Tree Flower (*Bombax ceiba* L.) Extract

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ABSTRACT

The red cotton tree flower (*Bombax ceiba* L.) is a local plant commonly found in Northern Thailand. It is used in Northern Thai cuisine and traditional Thai medicine. A previous study demonstrated the antioxidant properties of red cotton tree flowers in a formulated skincare product, specifically a sleeping mask gel. This study aimed to develop an anti-wrinkle facial skincare product containing red cotton tree flower extract. The flowers were extracted using 99% ethanol through maceration at room temperature. A 1.5% concentration of the extract was incorporated into the sleeping mask gel. An *in vivo* study was conducted to evaluate skin irritation and anti-wrinkle efficacy. Twenty volunteers aged 30–60 years (7 men and 13 women) participated in the study. The results showed no skin irritation among the volunteers, and the product effectively reduced wrinkles after one month of use. Additionally, it increased skin moisture within the first week of application. The volunteers expressed overall satisfaction with the product and reported no adverse reactions. This newly developed product not only highlights the effectiveness of red cotton tree flower extract but also demonstrates its commercial potential.

Keywords: Red cotton tree flower extract, *Bombax Ceiba*, Anti-wrinkle, Antioxidant, Sleeping mask gel

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Introduction

The red cotton tree (*Bombax ceiba* L.) belongs to the Malvaceae family and is commonly found in East and Southeast Asia. It is a perennial tree with brown and green bark covered in thorns. The tree produces red-orange flowers that bloom for 1–2 weeks before falling to the ground. Its young fruit is light green and gradually turns brown and dry upon maturation [1-2]. In Thailand, the red cotton tree is commonly found in the northern and central regions. The red cotton tree flower is edible and is a key ingredient in Northern Thai cuisine, where it is known as "Dok Gwew." The pollen from its flowers is also used in various Northern Thai dishes. In traditional Thai medicine, red cotton tree flowers are valued for their medicinal properties. Different parts of the tree have been used to treat various ailments, including gastritis, diarrhea, hemorrhoid wounds, skin inflammation, and irritation [3-6]. Several studies have investigated the bioactive compounds of red cotton tree flowers. Extracts obtained using water, ethanol, and other solvents have been analyzed for their total phenolic content (TPC) and total flavonoid content (TFC). Antioxidant activities were assessed using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) radical cation assay, and the ferric reducing antioxidant power (FRAP) assay. The findings revealed that ethanol extracts of red cotton tree flowers contained strong antioxidants due to the presence of flavonoids and anthocyanins, making them suitable for use in pharmaceutical products [2, 4, 7-8]. Additionally, another study demonstrated that methanolic extracts of red cotton tree bark exhibited antibacterial activity and contained alkaloids, tannins, glycosides, and terpenoids. Furthermore, research on red cotton tree flowers found that ethanol and water extracts had the highest polyphenol content, while ethyl acetate, hexane, and acetone extracts had the highest flavonoid content. Water extracts, on the other hand, contained the highest levels of condensed tannins [4, 9].

A previous study extracted red cotton tree flower using 99% ethanol and compared its DPPH radical scavenging ability with that of ascorbic acid. The results showed that the IC_{50} value of the red cotton tree flower extract was 0.41±0.01 mg/mL, whereas the IC_{50} value of ascorbic acid was 0.04±0.01 mg/mL, indicating that the antioxidant activity of the red cotton tree flower extract was lower than that of ascorbic acid. To develop a sleeping mask gel containing red cotton tree flower extract, a 100 g formulation was prepared, incorporating 1.5 g (1.5% w/w) of the extract. This amount was more than 100 times the IC_{50} value of the extract's antioxidant activity. The formulation underwent a stability evaluation using a heating-cooling test for six cycles, and its antioxidant activity was assessed. The results showed that the product had good stability, with no visible changes in physical appearance after the stability test. However, the remaining antioxidant activity (DPPH inhibition) significantly decreased ($p<0.05$), with a 48.10% reduction in DPPH inhibition ability. Despite this, the product retained 52.90% antioxidant activity, demonstrating continued effectiveness. Therefore, this formulation exhibited antioxidant properties and good product stability. This experiment confirmed that the sleeping mask gel containing red cotton tree flower extract retained antioxidant activity, making it a promising candidate for skincare applications [10-11].

Based on the aforementioned findings, this study aimed to evaluate the *in vivo* effects of a sleeping mask gel containing red cotton tree flower extract. The focus was on skin irritation tests and anti-wrinkle activity, assessed by volunteers aged 30–60 years. This research highlights the commercial potential of Thai local plant extracts for future developments in the skincare industry.

Materials and Methods

Materials

Red cotton tree flowers were sourced from a local area in Chiang Mai, Thailand. The fresh flowers used in this study were fully bloomed, with red-orange petals and intact carpels and stamens (Figure 1). Chemicals and reagents were obtained from the following suppliers: 99% ethanol was purchased from Union Science Co., Ltd., Thailand. Distilled water, 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, L-tyrosine, mushroom tyrosinase enzyme, kojic acid, and ascorbic acid were obtained from Sigma-Aldrich, Germany. Sodium lauryl sulfate (SLS), glycerin, PEG-240/HDI copolymer bis-decyltetradeceth-20 (Reshape Gel), niacinamide (Vitamin B3), panthenol (Vitamin B5), 1% sodium hyaluronate, glidant, disodium EDTA, and triethanolamine were sourced from Phitsanulok World Chemical Co., Ltd., Thailand.

Figure 1 Red cotton tree fresh flower.

Preparation of the red cotton tree flower extract

Fresh red cotton tree flowers were sun-dried at a temperature of 31–35°C for 48 hours. The dried flowers were then further dried in a hot air oven at 50°C for 3 hours. The dried samples were extracted using 99% ethanol at a 1:10 w/v ratio through maceration at room temperature for 48 hours. After maceration, the extract was filtered to remove any residue, and the resulting liquid was evaporated to dryness to obtain the crude extract.

Preparation of sleeping mark gel

The sleeping mask gel was formulated using water, glycerin, niacinamide (Vitamin B3), panthenol (Vitamin B5), 1% sodium hyaluronate, glydant, disodium EDTA, triethanolamine, PEG-240/HDI copolymer bis-decyltetradeceth-20, and 1.5% red cotton tree flower extract (w/w). All ingredients were blended using a cold process to ensure proper mixing and stability [11].

In vivo skin irritation test and anti-wrinkle activity

Ethical consideration

Approval for this human study was obtained from the Human Ethics Committee of the Faculty of Pharmacy, Chiang Mai University, Thailand.

In vivo skin irritation test

A skin patch test was conducted to assess skin irritation on the forearms of 20 volunteers. The volunteers' forearms were cleaned with distilled water prior to the test. The sample was placed in a Finn chamber patch, which was then applied to the forearm and secured for 48 hours. The Finn chamber was divided into three sections: Area A contained the sample product, Area B contained 2% SLS, and Area C contained distilled water (Figure 2). After 48 hours, the patch was removed, and skin erythema and edema were evaluated by taking photographs at 1, 24, 48, and 72 hours post-removal. The primary dermal irritation index (PII) was calculated using the following equation :

$$\text{PII} = \frac{[(\sum \text{erythema grade at 1/24/48/72 h} + \sum \text{edema grade at 1/24/48/72 h})]}{4 \times \text{number of subjects}}$$

The PII for the *in vivo* skin irritation test was determined based on the skin reaction values and the primary dermal irritation index, following the OECD guideline for primary dermal irritation/corrosion [12].

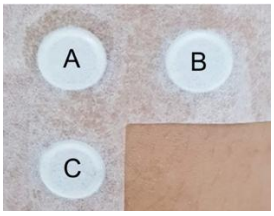


Figure 2 Positions for applying the patch test; (A) Sample, (B) 2% SLS, (C) Blank skin.

Table 1 The value of skin reaction.

Value	Skin reaction	
	Erythema	**Edema**
0	No erythema	No edema
1	Slight erythema	Very slight edema
2	Well-define erythema	Slight edema
3	Moderate or severe erythema	Moderate edema
4	Severe erythema and slight eschar formation	Severe edema

Table 2 The primary dermal irritation index.

Primary dermal irritation index	Classification of skin irritation
< 0.5	Non – irritation
0.5 – 2.0	Slightly irritation
2.1 – 5.0	Moderately irritation
> 5.0	Severely irritation

In vivo anti-wrinkle activity

The anti-wrinkle activity was evaluated by 20 volunteers aged 30 to 60 years. Volunteers were required to have no history of allergic skin reactions, no current treatments or medications, and no wounds or tattoos in the tested area. Initially, all volunteers cleaned their faces and allowed their skin to acclimate in a control room at 25°C for 30 minutes before starting the skin evaluation. Skin assessments were performed using the Visiometer® to measure wrinkles and the Corneometer® to assess skin moisture. Volunteers applied the product to their faces every night before bedtime for 1 month. After 7, 14, 21, and 30 days of use, their skin was retested using the Visiometer® and Corneometer®.

Statistical analysis

The data are presented as the mean ± standard deviation (SD). Statistical analysis was performed using paired t-tests, with a significance level set at 95% ($p < 0.05$).

Results and Discussion

The results of this experiment showed that the sleeping mark gel containing 1.5% red cotton tree flower extract (w/w), 2% w/v SLS, and bare skin had Primary Dermal Irritation Index (PII) values of 0.28, 2.45, and 0.21, respectively. These findings indicated that the sleeping mark gel with red cotton tree flower extract did not cause skin irritation in the volunteers, with results similar to the bare skin (negative control). In contrast, the irritation assessment for SLS was classified as moderately irritating. Therefore, based on these results, it can be concluded that the sleeping mark gel with red cotton tree flower extract did not cause skin irritation in the volunteers, as shown in Table 3.

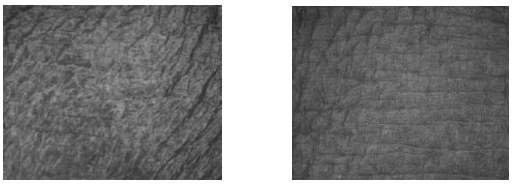
Table 3 The irritation assessment was conducted on 20 volunteers by observing swelling and redness on the skin of their upper arms before application and after removal of the test patch at 1, 24, 48, and 72 hours.

Number of volunteers	Positions of patch test	The skin after removal of the test patch			
		1 hour	24 hours	48 hours	72 hours
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					

Volunteers used the product on their faces daily for 1 month and their skin condition was assessed after 7, 14, 21, and 30 days of continuous use. Skin condition was evaluated using several tools: the Visiometer® to assess skin aging and the Corneometer® to measure skin moisture content. The Visiometer® is used to assess fine lines and wrinkles by capturing images of the skin on the forehead, outer corners of the eyes, and cheeks with an ultraviolet-light video camera (Camera Visioscan VC98). These images were then analyzed using the Visoscan FW program to determine wrinkle area (%Surface), wrinkle depth (Volume), and skin roughness (Roughness), as shown in Figures 3-5 and Table 4. Before applying the product, the

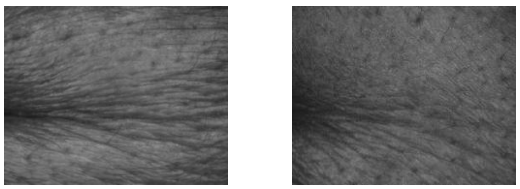
mean wrinkle area (%Surface) on the forehead, outer corners of the eyes, and cheeks were 472.78±23.27%, 481.86±10.11%, and 495.04±15.99%, respectively. After 30 days of product use, no statistically significant difference was observed in the mean wrinkle area on the forehead ($p > 0.05$). However, the mean wrinkle areas around the outer corners of the eyes and cheeks decreased to 420.98±13.47% and 422.24±12.67%. The mean wrinkle depth (Volume) before using the product was 64.51±6.05 mm^3 on the forehead, 78.96±10.12 mm^3 around the eyes, and 73.62±9.67 mm^3 on the cheeks. After 30 days of use, a statistically significant reduction was observed in wrinkle depth around the eyes and cheeks ($p < 0.05$), with values of 69.58±7.05 mm^3 and 65.10±13.05 mm^3, respectively. However, the mean wrinkle depth on the forehead increased, but not significantly ($p > 0.05$). Regarding skin roughness (Roughness), the mean roughness before using the product on the forehead, outer corners of the eyes, and cheeks was 46.80±2.01, 49.42±0.77, and 47.13±1.30, respectively. After 30 days of use, a slight decrease in skin roughness was observed on the forehead, but this change was not statistically significant ($p > 0.05$). The mean roughness values were 41.96±0.94 for the forehead and 39.45±1.12 for the cheeks.

Overall, the product showed a promising trend in reducing wrinkles and providing an anti-aging effect, with more noticeable results as the volunteers continued to use the product for over a month.



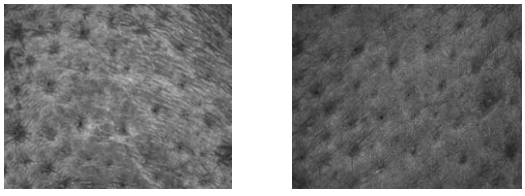
(A) (B)

Figure 3 Photographs of the forehead were taken before using the product (A) and after 30 days of use (B).



(A) (B)

Figure 4 Photographs of the outer corners of the eyes were taken before using the product (A) and after 30 days of use (B).



(A) (B)

Figure 5 Photographs of the cheek before were taken before using the product (A) and after 30 days of use (B).

Table 4 The area of wrinkles (%Surface), the depth of the wrinkles (Volume), and the roughness of the skin (Roughness) before and after using the product.

The area of skin	Date of skin condition assessment	Area of wrinkles (%Surface)	Depth of the wrinkles (Volume) mm^3	Roughness of the skin (Roughness)
Forehead	Before	472.78±23.27	64.51±6.05	46.80±2.01
	After 7 days	452.98±19.09	64.02±8.10	44.47±1.63
	After 14 days	465.89±22.12	61.62±9.51	46.47±1.61
	After 21 days	465.10±18.64	64.51±4.66	45.78±1.57
	After 30 days	467.66±15.25	72.04±7.09	44.56±0.97
Outer corners of the eyes	Before	481.86±10.11	78.96±10.12	49.42±0.77
	After 7 days	401.94±11.26*	58.29±6.19*	39.96±0.77*
	After 14 days	436.84±11.37*	65.69±6.55*	44.11±0.71*
	After 21 days	470.48±13.42*	69.49±7.84*	45.56±1.00*
	After 30 days	420.98±13.47*	69.58±7.05*	41.96±0.94*
Cheeks	Before	495.04±15.09	73.62±9.67	47.13±1.30
	After 7 days	424.08±11.13*	57.78±7.90*	40.27±1.36*
	After 14 days	454.05±15.99*	59.95±5.85*	43.30±1.29*
	After 21 days	427.30±16.02*	64.28±7.40*	42.07±0.91*
	After 30 days	422.24±12.67*	65.10±13.05*	39.45±1.12*

* statistically ($p < 0.05$) compared to before using the product

The results from the Corneometer® used to measure the volunteers' skin moisture before and after using the product showed that the mean skin moisture level before using the product was 50.3±1.6 a.u. After 7 days of continuous product use, the mean skin moisture increased to 57.7±1.9 a.u., showing a statistically significant improvement ($p < 0.05$) compared to baseline. After 14, 21, and 28 days of continuous use, volunteers experienced further statistically significant increases in skin moisture ($p < 0.05$). The values were 59.1±1.4 a.u., 61.1±1.1 a.u., and 65.5±1.3 a.u., respectively. These results demonstrate that the product effectively enhances skin moisture starting from the first week of use, as shown in Table 5.

Table 5 The skin moisture levels before and after using the product.

Date of skin condition assessment	Skin moisture (a.u.)
Before	50.3±1.6
After 7 days	57.7±1.9*
After 14 days	59.1±1.4*
After 21 days	61.1±1.1*
After 30 days	65.5±1.3*

a.u. = arbitrary corneometer units

* statistically ($p < 0.05$) compared to before using the product

The efficacy testing of the sleeping mask gel containing red cotton tree flower extract in volunteers was conducted using baseline values as a comparison instead of a control group. Although the study did not include a control group using a sleeping mask gel without red cotton tree flower extract, it used the baseline values of each volunteer before product application as a point of comparison. This method is widely accepted in cosmetic research and has been commonly applied in clinical studies [13-15], which used baseline comparisons to evaluate the effects of active ingredients in reducing wrinkles. Furthermore, the experimental results demonstrated a statistically significant reduction in wrinkles around the outer corners of the eyes and cheeks ($p < 0.05$) and an increase in skin moisture from the first week of use. Previous studies have reported that red cotton tree flower extract possesses antioxidant properties and enhances skin hydration, which aligns with the findings of this study. Although we did not directly compare the results with a control group using a sleeping mask gel without the extract, we used each volunteer's baseline as a reference. The obtained results are consistent with previous research indicating that red cotton tree flower extract exhibits biological activity in reducing wrinkles and increasing skin hydration [10]. For future studies, incorporating a control group using a sleeping mask gel without the extract could help further confirm the efficacy of the product.

Conclusions

The red cotton tree flower extract was fully incorporated into the sleeping mask gel product and tested on volunteers. The results demonstrated that the product is both safe and effective in reducing wrinkles and increasing skin moisture, providing protection against skin aging.

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