

Safety and Efficacy Evaluation on Combination of *Lansium domesticum* Fruit Extract and *Hibiscus rosa-sinensis* Flower Extract as Lightening Agent for Cosmetic

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Abstract—This study was designed to investigate the potency of a combination of *Lansium domesticum* fruit extract and *Hibiscus rosa-sinensis* flower extract (LHE) as cosmetic active ingredient. The *in vitro* tyrosinase inhibition method was used to evaluate the potency of LHE in inhibit melanin production thus potential to delivered lightening benefits for skin. The result was confirmed by 4-week *in vivo* lightening study on human skin. Prior to this *in vivo* efficacy study, safety evaluation was conducted using Single Closed Patch Test (SCPT) for skin and Hen's Egg Test – Chorioallantoic Membrane (HET-CAM) to evaluate the irritation at ocular level. LHE showed tyrosinase inhibition of 49.37%, which indicates that LHE has potency to be used as lightening active ingredients in cosmetics products. After 4 weeks of application in human skin, the efficacy test showed that LHE contained lotion base significantly increased skin moisture content and reduced its melanin index. Furthermore, safety evaluation of the lotion by SCPT and HET-CAM showed that it is safe for human skin and eyes. LHE is proven to be safe and effective as active ingredient in cosmetic.

Index Terms—cosmetic, *Hibiscus rosa-sinensis*, *Lansium domesticum*, lightening.

I. INTRODUCTION

Skin lightening is one of the most popular trends in skin care products. In Asia countries, such as Indonesia, Malaysia and China, a female beauty concept of bright and fair skin has created a demand of lightening cosmetics. The color of human skin is determined by a number of factors. Biosynthesis of the melanin pigment, namely melanogenesis, is the most important factor [1]. Hyperpigmentation is a common melanogenesis disorder caused by excess melanin production activated by the tyrosinase enzyme [2]. The interest of using natural sources as active ingredients of cosmetic is emerging. A number of natural ingredients with an inhibitory effect on melanin hyperpigmentation have been investigated and

patented, and some of them were developed as cosmetic products [3], [4].

Lansium or *lanzones*, known as *langsar* in Indonesia was originated in South East Asia and belong to family of *Meliaceae*. This plant bears fruit once a year which vary between areas but usually at the end of raining season. It traditionally used by indigenous female of Dayak in Borneo Island as talc powder to protect their skin from the sun. Meanwhile, *Hibiscus* is a flowering plant, native to East Asia and belongs to family *Malvaceae*. It is an ornamental plant and often planted as fence or hedge plant.

The aim of this study is to investigate the efficacy and safety of *Lansium* fruit and *Hibiscus* flower combination extracts (LHE) as cosmetic active ingredient.

II. EXPERIMENTAL

A. Material

Lansium aqueous glycerin extract, *Hibiscus* aqueous glycerin extract and base lotion were obtained from PT. Martina Berto, Tbk., Indonesia. Analytical grade of L-tyrosin (Sigma, USA), tyrosinase (Sigma, USA), sodium dihydrogen phosphate monohydrate (Merck, Germany), disodium hydrogen phosphate (Merck, Germany) and Kojic acid (Sigma, UK) were used for analysis of anti-tyrosinase activity.

B. Anti-Tyrosinase Activity

The anti-tyrosinase activity was determined according to the method of Vanni *et al.*, 1990 [5] using L-tyrosin solution as substrate and Kojic Acid as positive control. The extract or positive control was diluted in phosphate buffer and the L-tyrosine and tyrosinase solution were added. Each mixture was then shaken and incubated at 37 °C for 1h before it was analyzed spectrophotometrically at 475nm. The percentage of

tyrosinase inhibitor can be calculated using the following equation:

$$\% \text{ tyrosinase Inhibitor} = \frac{A_b - (A_s - A_o)}{A_b} \times 100 \quad (1)$$

where A_b is absorbance of blank sample, A_s is absorbance of sample with tyrosinase and A_o is absorbance of sample without tyrosinase.

C. HET-CAM

This study was conducted to assess the eye irritation properties of cosmetic formulations and ingredients. HET-CAM was conducted according to the method by Luepke, 1985 [6]. The lotion contains LHE was applied on the fresh fertile white Leghorn eggs and left for 20 seconds. The membrane was then evaluated for five minutes for any appearance of hyperemia, hemorrhage and opacity.

D. Single Closed Patch Test

This study had a randomized and controlled design. Fifty-one healthy female subjects, age between 18 to 55 years old, with normal to dry skin type, were recruited for the study. These subjects must not applied skin care products on their back other than test products provided by study personnel one week prior and during observation. Moreover, they also had to sign informed consent which stated they understand and agree to all testing condition including the benefit they will accept and the risk they may be experienced.

The base lotion contains LHE was placed on Finn chamber (SmartPractice, USA), then applied at the subject's back skin. They were prohibited to wash their back or performing any activities that may result excessive sweating or exposing themselves to sunlight and/or UV radiation during 24-hour application. The patch was removed after 24 hours, then a series of evaluations were performed by trained evaluator after 1, 24, 48 and 72 hours for any skin reactions such as allergies and irritation. The subject who experienced skin reactions were graded at the end of observation based on scale according to the International Contact Dermatitis Research Group (ICDRG) [7].

E. Efficacy Evaluation

This study had a randomized and controlled design. Twenty eight healthy female subjects, age between 18 to 35 years old, with normal to dry skin type, were recruited for the study. These subjects must not applied skin care products on their face other than test products provided by study personnel one week prior and during observation. Moreover, they also had to sign informed consent which stated they understand and agree to all testing condition including the benefit they will accept and the risk they may be experienced.

The participated subject must not pregnant or in lactation period, has allergic history with any cosmetic products, using medicine or cosmetic either oral or topical which can interfere the observation.

After taking baseline measurements, the lotion contains 1% of each, *Lansium domesticum* fruit extract and *Hibiscus rosa-sinensis* flower extract was applied on face skin, twice daily in the morning and evening for duration of 4 weeks. Reports of adverse events were collected throughout the study.

Skin moisture content and skin melanin index were measured by Corneometer CM 820 (Courage Kazaka, Germany) and Mexameter MX 16 (Courage Kazaka, Germany), respectively. The measurements were taken after 2 and 4 weeks of test product application. Statistical analysis was performed using the paired Student's *t*-test where *p*-values of less than 0.05 were considered statistically significant.

III. RESULT

A. Anti-Tyrosinase Activity

The result showed that Lansium extract and Hibiscus extract at each at concentration of 2% gave the tyrosinase inhibition of 29.36% and 25.74%, respectively. Combination of both extracts gave a tyrosinase inhibition of 49.37%, higher than each of the single extract, as can be seen at Fig. 1. This shows that combination of Lansium and Hibiscus extract gave positive synergism.

It is reported that Lansium fruit and Hibiscus flower contains phenolics and flavonoids, which contribute to inhibit the tyrosinase activity [8]-[10]. Tyrosinase inhibition of LHE as much of 49.37% indicates that the extract combination can be used as active ingredients in cosmetics as lightening agent. Therefore the efficacy *in vivo* was investigated to prove this lightening activity. Prior to that, safety evaluation was conducted.

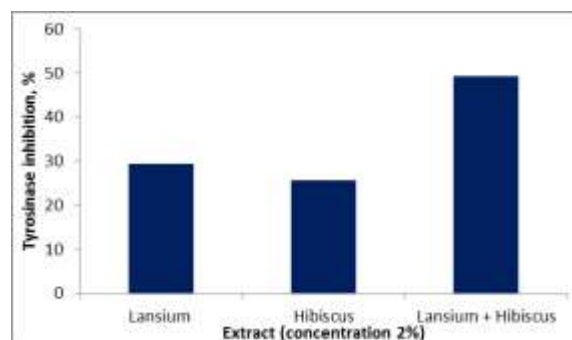


Figure 1. Tyrosinase inhibition of *Lansium* and *Hibiscus* extract.

B. Safety Evaluation

The HET-CAM revealed that the base lotion contains LHE caused no irritation at the ocular level as shown at Table I. Meanwhile, sodium lauryl sulfate as positive control, which known as surfactant found in soap or shampoo caused strong irritation with hemorrhage.

From the SCPT, which involved 51 subjects aged between 20-53 years old, it was found that the application of base lotion contains LHE at concentration of 2% did not cause any irritation or allergic reaction. None of subject experienced any skin reactions either irritation or allergic reaction as can be seen at Table II.

TABLE I. RESULTS OF HET CAM OF BASE LOTION CONTAINS LHE

Sample	Mean Irritation index	Hemorrhage
Negative control: 0.9% NaCl	0	No
Positive Control: 0.5% Sodium Lauryl Sulfate	12	Yes
Base lotion contains 2% LHE	0	No

TABLE II. RESULTS OF SCPT OF BASE LOTION CONTAINS LHE

Skin Response	Subject number person	Percentage of total subject (%)
Irritation	0	0
Allergic reaction	0	0

C. Efficacy Evaluation

Efficacy evaluation was conducted to measure skin moisture content and skin melanin index. The results were shown at Fig. 2. Skin moisture content increased significantly by 9.9 and 8.3 units respectively after two and four weeks of application ($p < 0.001$). Meanwhile, the melanin index significantly reduced by 0.9 and 3.5 after two and four weeks of application ($p < 0.05$), respectively.

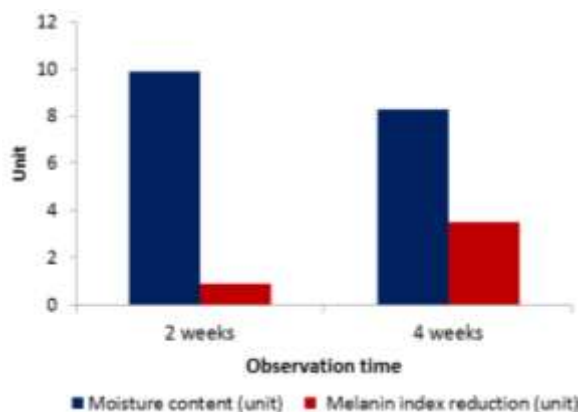


Figure 2. Efficacy evaluation of lotion contains *Lansium* and *Hibiscus* extract (N=28).

CONCLUSION

The base lotion contains combination of *Lansium* fruit extract and *Hibiscus* flower extract tested at 2% concentration has potential lightening activity in cosmetics. Furthermore, this extract is also has a potency as moisturizing agent. The extract at concentration of 2% is safe as active ingredient in cosmetic.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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