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Research Article

## Skin whitening efficacy of *Gnetum gnemon* L. seed extract

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### ABSTRACT

*Gnetum gnemon* L. (Melinjo) seed extract (MSE) is potential as skin-whitening agent because it contains trans-resveratrol and its derivatives, to inhibit tyrosinase in melanogenesis process. Using MSE in cosmetic products will be challenging due to resveratrol chemical instability and bioavailability in the skin. Many cosmetic products have been developed using lipid particle technology to improve their limitation. The objective of this research was to examine the skin safety and whitening efficacy of MSE-loaded lipid particle gel in healthy human subjects.

**Materials and Methods:** Single occlusive closed patch test for 24 h was used as the skin irritation analysis. Irritation responses were graded after patch removal and compared to the control for evaluation.

**Results:** The result showed the test product did not induce skin irritation effect. The skin melanin index was statistically significant decreased ( $P < 0.05$ ) after 28 days of application the test product, with the averaged by 3.50%, and skin melanin index changed by increase 0.75% in the control group.

**Conclusion:** Application MSE-loaded lipid particle gel can brighten the skin, without cause irritation under normal conditions of use.

**Abbreviations Used:**  $\alpha$ -MSH:  $\alpha$ -Melanocyte Stimulating Hormone; MITF: Microphthalmia-Associated Transcription Factor; MSE: Melinjo Seed Extract.

**Keywords:** Gel, *Gnetum Gnemon*, Melinjo Seed Extract, Resveratrol, Skin Whitening

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### 1. INTRODUCTION

Melanin contributing to human skin color, especially eumelanin, is the major pigment that has a primary function of protecting the skin from damage caused by ultraviolet (UV) exposure such as photoaging or photocarcinogenic.<sup>1</sup> Melanocytes cell in the basal layer of epidermis contains melanosome. The melanosome function is a synthesis, storage, and transfer of melanin to the outermost layer of skin (keratinocytes).<sup>2</sup> Melanogenesis could be induced by UV radiation through stimulated the secretion of a  $\alpha$ -melanocyte-stimulating hormone that would be activated micro-ophthalmia-associated transcription factor (MITF). MITF would lead to up regulation of tyrosinase, the primary enzyme in melanogenesis.<sup>3</sup>

Tyrosinase involved in two different oxidation reactions to melanin synthetic pathway. The first step is the hydroxylation of L-tyrosine to L-DOPA, and the second step is oxidation L-DOPA to dopaquinone. From dopaquinone, the melanogenesis pathway is divided. Dopaquinone

spontaneous conversion to dopachrome and through the tyrosinase-related protein 1 and 2 turns dopachrome into eumelanin. In the other, dopaquinone also conjugated with cysteine or glutathione to form pheomelanin. Eumelanin is a black-brown melanin, whereas pheomelanin is a yellow-red melanin.<sup>4</sup> UV exposure is one of the major causes of esthetic skin problem such as skin pigmentation. However, it causes abnormal activity of tyrosinase that involved excessive of melanin. Because some people prefer white skin, they always find cosmetics to reduce pigmentation. Thus, inhibition of tyrosinase and melanogenesis will be important as a target for skin-whitening agent.<sup>5,6</sup>

*Gnetum gnemon* L. belongs to the genus *Gnetum* in Gnetaceae family. This plant is widely cultivated in Southeast Asia, especially in Indonesia; melinjo is the familiar name for the plant. Melinjo seeds in ripe fruits are popular as a vegetable consumed in soup or made crackers (local name: emping) with a slightly bitter taste.<sup>7</sup>

Resveratrol (a polyphenolic compound group of a stilbenoid) from melinjo seeds must be considered as one of a natural source of resveratrol with high bioavailability and safety. Melinjo seed extract (MSE) contains abundant in resveratrol derivatives, in the form of resveratrol monomer derivative, which is trans-resveratrol (3, 5, 4-trihydroxy-trans-stilbene), trans piceid, and isorhapontigenin; and resveratrol dimer derivative, which is gnetin C, gneunoside A, gneunoside D, gneunoside C, and gnetin L.<sup>8,9</sup> Gnetin C, gneunoside A, and gneunoside D are major constituents, while trans-resveratrol is a minor constituent of MSE.<sup>10</sup>

Resveratrol derivatives in MSE revealed beneficial pharmacological effects. That is known as lipase and  $\alpha$ -amylase inhibitors, antimicrobial,<sup>10</sup> antioxidant against free radicals,<sup>11</sup> immunostimulator,<sup>12</sup> inhibition on tumor angiogenesis,<sup>13</sup> decreased serum uric acid and increased high-density lipoprotein.<sup>14</sup> MSE has had cosmeceutical properties. Previously on the *in vitro* and *in vivo* study, trans-resveratrol potent inhibition of tyrosinase and suppress melanogenesis activity,<sup>15,17</sup> as good as the work of Gnetin C.<sup>18</sup> Therefore, MSE might be potential use as new skin-whitening agent.

Trans-resveratrol is restricted to formulate in general cosmetic product. Trans-resveratrol unstable because susceptible to degradation by the light,<sup>19</sup> which it would be changed trans-isomer resveratrol to cis-isomer, that less biologically active.<sup>20</sup> Moreover, resveratrol is poor solubility in water, which influences absorption and lowering penetration of resveratrol to the skin. Their limitation could be enhanced when resveratrol inclusion into lipid system.<sup>21</sup> Lipid particles in the topical cosmetic formulation have shown to be helpful to improve stability and biological activity by protecting effect, including water solubility to increasing penetration through stratum corneum by skin hydration that means to enhance the bioavailability of resveratrol in the skin.<sup>22,23</sup>

The aim of this current study was to examine the whitening effect of MSE-loaded lipid particle gel in the human healthy skin.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Cutina® glyceryl monostearate (GMS) V and Cremophor® A 6 (cetareth-6 and stearyl alcohol). Carbopol® Ultrez 20 polymer (Lubrizol), propylene glycol (Dow Chemical Ltd.), sodium metabisulfite, potassium sorbate, Na<sub>2</sub> EDTA (Nobel Chemical), and sodium hydroxide were used in the study. MSE contains trans-resveratrol of 3.3 mg/g.

### 2.2. Formulation of lipid particle gel

The formulation of MSE-loaded lipid particle system was GMS (10%), CS25 (4%), A6 (0.8%), MSE (10%), and water (75.2%). The lipid particle dispersion was prepared using the high-shear homogenization and hot-melted technique. After all the materials melted and mixed, added MSE, and then homogenized at 1250 rpm for 20 min and 30,000 rpm for 5 min (H04 Edmund Buhler GmbH, Germany). Characterization of MSE containing resveratrol-loaded lipid particles was determined by particle size, potential zeta, and entrapment efficiency. This method based on a previously reported method, with some modifications.<sup>24,25</sup>

The gel formulation was carbomer (0.4%), propylene glycol (10%), sodium metabisulfite (0.1%), potassium sorbate (0.15%), Na<sub>2</sub> EDTA (0.01%), 18% sodium hydroxide solution (0.5%), perfume (0.1%), MSE-loaded lipid particles (10%), and added water *ad* 100%. The gel was prepared with mechanically stirring by dispersed gel base into the water and added MSE-loaded lipid particles. Analysis amount of the MSE containing resveratrol in the product was performed using high-performance liquid chromatography (HPLC).

### 2.3. The products

The test product was MSE-loaded lipid particle gel as the active ingredient. The control product was gel base with the same formulation as the test product, without the active ingredient.

### 2.4. Human participants

All the women volunteers, who met the inclusion and exclusion criteria Table 1, must sign an informed consent to participate in this study.

**Table 1: Inclusion, exclusion, and dropout criteria for the participants**

<b>Inclusion Criteria</b>	-Healthy Women aged 20 +
	- Willing to signed Informed consent to become a volunteer, after informed the purpose and any information of the study
	- Willingly stop used cosmetic products in the around test area, starting from 1 week before and during the test
	- Willing to cooperative during the study
<b>Exclusion Criteria</b>	-Pregnancy or breastfeeding
	-Around test area presence tattoos, scars, sunburns or uneven skin tones and excessive skin sunlight exposure.
	-Anyone used any drug that affects skin reaction
	-Anyone with a chronic disease, infectious skin disease or any skin disease.
<b>Dropout Criteria</b>	-Smoking, alcoholic or drug user
	-Show of allergy reaction to the products during the test
	- No used the product
	- No came to the research centre for examination

### 2.5. Skin irritation test

Skin irritation test of the products was performed using patch test. Human patch tests were conducted for 24 h with an occlusive patch. Thirty-eight women between 20 and 40 years old participated in this study. The products were dropping 20  $\mu$ l in a Finn Chamber tape (Smart Practice, Arizona, United States). It was applied to the upper arm as a

test side. The skin irritation response of patched side was observed and graded at 30 min, 24 h, and 48 h after patch removal by an experienced assessor.<sup>26</sup>

The grading criteria for human skin irritation are shown in Table 2. Skin responses were evaluated, and the score was calculated according to the formula.<sup>27</sup>

**Table 2: Grading criteria for skin irritation**

Grade	Clinical Response
0	Negative reaction
1	Slight erythema, spotty or diffuse
2	Moderate erythema
3	Intense erythema with edema
4	Intense erythema with edema and vesicles

$$\text{Irritation Score (R)} = \frac{\sum (\text{grade} \times \text{number of responders})}{4 \times \text{total subjects}} \times 100 \times \frac{1}{2}$$

The score of irritation response was converted to human primary irritation index criteria for cosmetic products from human irritation patch test Table 3.<sup>27</sup>

**Table 3:** Human primary irritation index

Irritation Source (R)	Criteria
0.00 < R < 0.87	No/Slight irritation
0.88 < R < 2.42	Mild Irritation
2.42 < R < 3.44	Moderate Irritation
R > 3.44	Severe Irritation

## 2.6. Skin-whitening test

The study was a single-blind clinical trial with the inter subject design. Twenty-five women between 20 and 40 years old were enrolled to participate in this study. The participants received a test and a control product that was applied once daily at night before sleep to the left or right of the upper arms for 28 days. The side for applying the product was measuring 6 cm x 6 cm. Every participant received a diary for monitoring their compliance and recording adverse events during the test. Participants came to the research center for measurement of melanin index on both sides of the upper arm. Their melanin index was analyzed using a Mexameter (Cutometer® dual MPA 580, Courage + Khazaka electronic GmbH, Germany). The evaluation was performed in the room, where the temperature was maintained at 22°C ± 2°C. The measurement was conducted at baseline before used the product and at treatment weeks 2 and 4. Each measurement is taken three times, and the average values were used for the analysis.

## 3. RESULTS

### 3.1. Melinjo seed extract-loaded lipid particle gel

Lipid particles system was prepared by the dispersed MSE into the melted GMS (lipid phase) and mixed with the hot water surfactant (water phase) to form the emulsion. Characterization of the MSE-loaded lipid particles showed that the higher average particle size was 794.6 ± 201.5 nm. The potential zeta value was 62.56 mV, which means that the lipid particle formula was stable when stored, and there is no tendency for aggregation or gelation phenomena. A good potential zeta value ranges from Z > +25 mv or Z < -25 mv.

The entrapment efficiency of MSE containing resveratrol in lipid particle system was evaluated to determine the amount of encapsulated resveratrol. Lipid system synthesis used centrifugation method. The concentration of resveratrol in the lipid system is as total resveratrol, and the filtrate of centrifugation result is as free resveratrol (not trapped in the system). They were measured using HPLC and calculated by the equation as below:

Entrapment Efficiency =

$$\frac{(\text{total resveratrol} - \text{free resveratrol})}{\text{total resveratrol}}$$

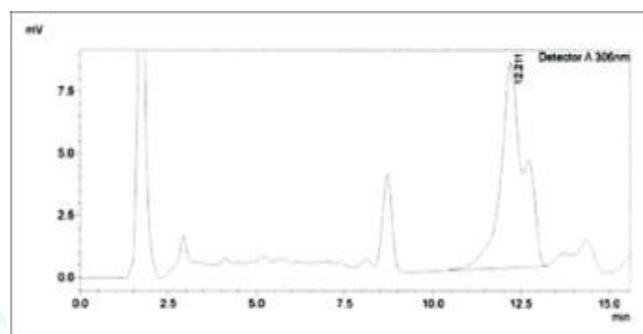
The entrapment efficiency result was 89.46% ± 0.04%. The result was indicated MSE incorporation into lipid system as

well. The good solubility of MSE in the lipid is one of the factors determining the high entrapment efficiency. GMS as a solid lipid matrix material is monoglyceride lipid that promotes enhanced MSE solubilization. Combination type of surfactant and lipid also would give a good result to enhanced MSE entrapment efficiency.

Furthermore, the gel was easily absorbed into the skin and did not leave a sticky impression when applied on the hand.

### 3.2. High-performance liquid chromatography

Final analysis of the resveratrol contained in products of MSE-loaded lipid particle gel was evaluated using HPLC. The HPLC system was isocratic, volume injection 20 µL. The stationary phase was a C-18 column (150 mm × 4.6 mm I. D., 5 µm) with temperature column 25°C. The mobile phase consisted of 75% water and 25% acetonitrile adjust pH 3. The flow rate was 1 mL/min. The UV detector was set at 306 nm. The final product contains 0.008% resveratrol (percent recovery: 155.10%). The HPLC profile is shown in Figure 1.



**Figure 1:** High-performance liquid chromatography chromatogram for resveratrol of the products used in this study

## 4. DISCUSSION

Resveratrol is polyphenolic compound found in various plants, including *G. gnemon* L. On previous *in vitro* study to support these study result, resveratrol from melinjo seed has shown inhibit tyrosinase on melanin synthesis in murine. Moreover, resveratrol also inhibits tyrosinase on human melanocytes, and guinea pig skin induced pigmentation by UV-B radiation. Resveratrol could act as a whitening agent due to inhibit melanogenesis activity through reduced MITF factor and tyrosinase promoter activities that would down-regulation tyrosinase, the effect on tyrosinase maturation and melanin synthesis.

The resveratrol is known for poor water solubility, which is influence skin absorption. The way to improve skin penetration of resveratrol with lipid particles has been investigated by Sun *et al* Lipid particles are a suitable system for hydrophobic compound incorporation. The lipid particle, especially solid lipid matrix, could enhance the penetration into the skin. The amount of active compound is retained in the outer skin surface reduced. Meanwhile, the lipid particles have an occlusion effect, which could reduce transepidermal water loss and helps to increase the penetration of active ingredients into the skin. Thus, MSE containing resveratrol is lipophilic nature ingredient suggesting a preferential into particle lipid matrix to improve properties of resveratrol as an active.

In the cosmetic industry, the safety and efficacy study is important to the product. This study has been investigated skin irritation potentials of the products. And no one of the volunteers that has skin irritation after skin patch test during 48 h. The criteria for classification of primary irritation index of human skin are used in the cosmetic products based on

the actual usage. A cosmetic compound is a mixture of various chemical ingredients in the formula, which could be potential as an irritant and trigger skin irritation, depending on the length of exposure time and the amount of use.

There has been no clinical trial study to examine the effects of MSE as topical skin whitening. Based on this study, the test product could give good result to support the claim of human skin-whitening effect of *G. gnemon* seed extract containing resveratrol. The application gel containing MSE-loaded lipid particles resulted in a significant reduction melanin index on the weeks 2 and toward lower melanin index afterward until weeks 4. This effect is probably due to the trans-resveratrol content and their derivatives, such as gnetin. However, the lipid particles as a delivery system of an active ingredient may also help to improve biological activity and bioavailability by increasing skin penetration to the target side.

## CONCLUSION

In conclusion, MSE which contains resveratrol has the potency as a cosmetic ingredient for the skin. Cosmeceuticals designed of MSE-loaded lipid particle gel exhibited a beneficial effect for skin whitening, without induced skin irritation.

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