

Study on the Safety and Efficacy of Indonesian Combination Plant Extract in Cosmetics

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ABSTRACT

Objective: To look for antioxidant, lightening and anti aging effects from combination of Indonesian plant consist of *Centella asiatica*, *Camellia sinensis* and *Glycine max* extract in propanediol (CCGE). **Methodology:** The study was carried out using DPPH scavenging activity for antioxidant effect and anti tyrosinase activity for lightening effect. The efficacy test on volunteers was performed using Mexameter MX 16 and Cutometer MPA 580 to measure skin melanin index, elasticity and firmness respectively. The dermatological safety test was conducted using Repeated Opened Patch Test (ROPT) and Single Closed Patch Test (SCPT), while HETCAM test was performed to evaluate irritation at ocular level. **Result:** At the concentration of 2%, CCGE has DPPH scavenging activity and anti tyrosinase activity of 70.56% and 52.76% respectively compared to ascorbic acid and kojic acid. The efficacy test showed that CCGE significantly increased skin elasticity and firmness after two and four weeks of application compared to base, but showed no significant effect on skin lightening. The dermatological safety test showed that CCGE did not cause any irritation or allergic skin reaction at the concentration of 5% for the ROPT and up to 3% for the

SCPT. Meanwhile at the concentration of 5%, CCGE could cause allergic reaction in 4.0% of all subjects. The HETCAM test showed that CCGE is not irritant at the ocular level. **Conclusion:** These results concluded that CCGE is proven to be safe for human skin as well as at ocular level and could be used as cosmetic active ingredient for anti aging.

Key words: *Centella asiatica*, *Camellia sinensis*, *Glycine max*, Antioxidant, Lightening, Anti aging, Cosmetic.

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INTRODUCTION

Natural cosmetic ingredients have shown tremendous growth in recent years,¹ as the demand for naturally derived active ingredient continues to increase globally along with the trend “back to nature”. Compare to cosmetic products with synthetic chemicals, natural cosmetic products are safer to use, have relatively less side effect, and more compatible with all skin types.²

Indonesia is the biggest archipelago³ in the world with an extraordinary biodiversity which been providing the populations with uncountable sources of ingredients that are used traditionally to treat ailments and health discomforts as well as for beauty care. They used various parts of local plants and trees, from roots, tubers, leaves, flowers, fruits, seeds, and even barks and twigs. This indicates that actually the concept of “live with nature” has long been embraced as Indonesian philosophy of life since ancient time.

The aim of this study is to examine the safety and efficacy of a combination of Indonesian plant extracts for cosmetic use. Several aspects should be considered when utilizing botanical materials in cosmetic, such as, the quality of the plant materials, process, biological activity, and safety consideration.

MATERIAL AND METHODS

MATERIALS

Plants materials

Gotu kola (*Centella asiatica*) dried herb, tea (*Camellia sinensis*) dried leaf and soybean (*Glycine max*) dried seed obtained from a community plantation on a Javanese island were used for this study. A taxonomical study was conducted by literature.

Materials and reagents

To prepare plant extract, the following materials were used: ethanol (Molindo Raya), demineralised water (Martina Berto, PT), propanediol (DuPont Tate & Lyle Bioproduct), and phenoxetol (Clariant). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (sigma) and methanol (Merck) were used in measuring the antioxidant activity with L(+) ascorbic acid (Merck) as positive control. L-tyrosine (Sigma), tyrosinase (Sigma), sodium dihydrogen phosphate monohydrate (Merck), and disodium hydrogen phosphate (Merck) were used in measuring the anti tyrosinase activity with Kojic acid (Sigma) as positive control.

Equipment

Electronic balance (Mettler Toledo), rotary evaporator (Buchii), spectrophotometer UV-16001 (Shimadzu), waterbath (Mettmert), loupe for observing the skin condition in the dermatological safety test. Mexameter

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MX 16 was used for measuring skin melanin index, while Cutometer MPA 580

METHODS

In-Vitro Eye Irritation & Dermatological Safety Testing

Material

Fresh fertile white leghorn eggs were used in *In-vitro* eye irritation testing and finn chamber with tape as tools to apply extract on human skin on dermatological safety testing.

Preparation of Extract

Gotu kola (*Centella asiatica*) dried herb, tea (*Camellia sinensis*) dried leaf and soybean (*Glycine max*) dried seed was extracted in ethanol. This extract was evaporated into dryness using rotary evaporator in vacuum. Following that, dried extract was diluted in propanediol to obtain the final extract (CCGE).

Antioxidant Activity⁴

The antioxidant activity was determined according to the method of Shimada *et al.* using DPPH radical scavenging activity 3). 0.2 mL of CCGE in methanol and L (+) ascorbic acid in demineralized water was added to freshly-prepared methanolic solution of DPPH. Each mixture was then shaken and incubated at room temperature for 30 minutes before it was spectrophotometrically measured at 517 nm. The percentage of DPPH scavenging activity of each plant extract and L (+) ascorbic acid is expressed by:

$$\% \text{ Scavenging activity} = 1 - \frac{\text{test sample absorbance}}{\text{blank sample absorbance}} \times 100$$

Anti Tyrosinase Activity⁵

The whitening activity was determined *in vitro* according to the method of Vanni *et al.* using L-tyrosine solution as the substrate 4). Two mL of CCGE and kojic acid solution in phosphate buffer was added with 0.5 mL L-tyrosine solution and 0.5 mL tyrosinase solution or 0.5 mL phosphate buffer. Each mixture was then shaken and incubated at 37°C for 60 minutes before it was spectrophotometrically detected at 475 nm. The percentage of tyrosinase inhibition rate of CCGE and kojic acid is expressed by:

$$\% \text{ tyrosinase inhibition rate} = \frac{Ab - (At - A0)}{Ab} \times 100$$

where Ab is the absorbance of blank sample, At is the absorbance of sample with tyrosinase and A0 is the absorbance of sample without tyrosinase

Dermatological Safety Test⁶⁻⁸

Fifty (50) selected female subjects according to below criteria were recruited for this study.

Inclusion Criteria: The subjects who participated in this study had to be 18–54 years old with healthy and normal and/or sensitive skin. Prior to the study, they had to sign informed consent form that contains information regarding the study including the benefits and risks for them that may occur during the study. They also had to refrain from using any kind of products on their back skin except products that part of the study, one week before and during the study.

Exclusion Criteria

Subjects with skin and/or medical problem cannot be enrolled in the study. Those subjects with pregnancy, on lactation period, and/or using

oral/topical application of medicine that can influence the skin condition were also excluded.

Repeated Opened Patch Test (ROPT)

CCGE with concentration of 5% was applied on to the skin area of the back. During application, the area was left unwashed. The skin reactions were then evaluated by trained assessor after 24 hours, 48 hours and 72 hours after application. The reactions observed were skin irritation and allergies, objectively as well as subjectively.

Single Closed Patch test (SCPT)

CCGE with concentration of 1%, 3% and 5% were applied each under occlusive patch on the suitable area of the skin on the back. During application, the area was left unwashed. The patches were removed after 24 h of application. The skin reactions were then evaluated by trained assessor after 1 hour, 24 h, 48 h and 72 h after patches removal.

Evaluation Method

The skin reactions were evaluated based on duration and the severity level of the reaction. The observed phenomena were scored according to scale developed by the International Contact Dermatitis Research Group

HETCAM test⁹

To evaluate the irritation level of CCGE to mucous membrane, HETCAM test was conducted according to the method of Luepke. 5% of CCGE in demineralised water was applied onto the chorioallantoic membrane and left in contact for 20 seconds. The membrane was then evaluated for 5 minutes for any appearance of hyperemia, hemorrhage, and opacity.

Efficacy Evaluation¹⁰

The whitening, and elasticity and firmness effect of CCGE and base were evaluated clinically. Twenty- eight selected female subjects were involved. They were recruited according to below criteria.

Inclusion Criteria: The subjects who participated in this study has to be 18-35 years old with healthy and normal to dry skin. Prior to the study, they had to sign an informed consent form that contains information regarding the study including the benefits and risks for them that may occur during the study. They also had to refrain from using any kind of products on their face except products that part of the study, one week before and during the study.

Exclusion Criteria: Subjects with skin and/or medical problem cannot be enrolled in the study. Those subjects with pregnancy, on lactation period, and/or using oral/topical application of medicine that can influence the skin condition were also excluded.

Evaluation on Skin Melanin Index

Initial/baseline evaluation on skin melanin index was conducted by using Mexameter MX 16. Then, according to randomization scheme CCGE and base were applied on each facial skin twice a day for 4 weeks. Other evaluations were taken place at 2 weeks after application.

Evaluation on Skin Elasticity & Firmness

Initial/baseline evaluation on skin elasticity & firmness was conducted by using Cutometer MPA 580. Then, according to randomization scheme CCGE and base were applied on each facial skin twice a day for 4 weeks. Other evaluations were taken place at 2 weeks after application.

RESULTS AND DISCUSSION

Antioxidant Activity

The result showed that CCGE at concentration of 2% had DPPH scavenging activity of 68.37% with 70.56% relative potency to ascorbic acid as the reference substance. This result indicate that CCGE has antioxidant activity against free radical of DPPH and therefore it has a potency to be antioxidant agent.

Anti Tyrosinase Activity

The test showed that CCGE at concentration of 2% had tyrosinase inhibitory activity with inhibition rate of 52.06% and potency ratio of 52.76% compared to kojic acid as the reference substance. This result indicate that CCGE has anti tyrosinase activity and therefore has potency to be whitening agent.

Dermatological Safety Test

In the dermatological safety test, fifty subjects aged between 18-54 years old had completed the study, which consisted of 22 subjects with sensitive skin and 28 subjects with normal skin. The complete result can be seen on Table 1. From the ROPT, it was shown that CCGE did not cause any skin reaction. From SCPT, CCGE did neither cause any irritation nor allergic skin reaction at the concentration of 1% and 3%. However, at the concentration of 5%, it did not cause any irritation in all subjects

Table 1: Result of Safety Evaluation on CCGE in human skin (n=50)

Method(s)	Concentration	Skin Reaction			
		Irritation		Allergic	
		N	%	N	%
ROPT	5.0%	0	0.0	0	0.0
	1.0%	0	0.0	0	0.0
SCPT	3.0%	0	0.0	0	0.0
	5.0%	0	0.0	2	4.0

N: Number of subject with positive response

Table 3: Influence of CCGE on skin lightening compared to baseline (n=28)

Product	Skin Melanin Index (unit)		
	Baseline	After 2 weeks	After 4 weeks
CCGE	524.19 ± 3.5	520.62 ± 3.3ns	521.29 ± 3.4 ns
Base	532.9 ± 3.7	529.8 ± 3.7	529.9 ± 3.4

ns: no significant difference to base was observed (p>0.05)

and only caused allergic in 4.0 % of the subjects. As from subjective assessment, none of the subject experiences any uncomfortable sensation.

HETCAM test

The HETCAM test revealed that at the concentration of 5% CCGE in demineralised water did not cause any irritation at the ocular level (Table 2).

Evaluation on Skin Melanin Index

The evaluation of Mexameter showed that CCGE significantly reduce skin melanin index by 3.6 units and 2.9 units after two weeks and four weeks of application respectively. However, these values were not significant to its base (Table 3).

Evaluation on Skin Elasticity and Firmness

The evaluation using Cutometer showed that application of CCGE increase skin firmness significantly after two weeks and four weeks by 6.2 percent and 18.1 percent (Table 4). These values were also significantly higher than its base. The application of CCGE also increase skin elasticity significantly by 5.6 percent and 10.3 percent respectively, which were also significantly higher than its base (Table 5).

Table 2: Result of HETCAM Test on CCGE

Sample	Mean Irritation Index (N)*	Haemorrhage
Negative control: 0.9% NaCl	0	No
Positive control: 0.5% Sodium Lauryl Sulfate	12	Yes
Testing product: CCGE in demineralized water (5%)	0	No

* : N < 1 (practically non irritant); 1 < N < 5 (slightly irritant); 5 < N < 9 (moderately irritant); N > 9 (strongly irritant)

Table 4: Influence of CCGE on skin firmness compared to baseline (n=28)

Product	Skin Firmness (unit)		
	Baseline	After 2 weeks	After 4 weeks
CCGE	0.3645 ± 0.011	0.3419 ± 0.014*	0.2987 ± 0.012*
Base	0.1858 ± 0.010	0.1842 ± 0.089	0.2088 ± 0.013

*: significantly winning/losing from baseline at p< 0.05

Table 5: Influence of CCGE on skin elasticity compared to baseline (n=28)

Product	Skin Elasticity (unit)		
	Baseline	After 2 weeks	After 4 weeks
CCGE	0.7137 ± 0.018	0.7535 ± 0.015*	0.7874 ± 0.012*
Base	0.8674 ± 0.014	0.8653 ± 0.013	0.8924 ± 0.013

*: significantly winning/losing from baseline at p< 0.05

CONCLUSION

Combination of *Centella asiatica*, *Camellia sinensis*, and *Glycine max* extract (CCGE) is proven to be safe on skin application as well as at ocular level. *Centella asiatica*, *Camellia sinensis*, and *Glycine max* extract (CCGE) as combination extract can significantly improve skin firmness and elasticity which are related to aging.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest.

ABBREVIATION USED

CCGE: *Centella asiatica*, *Carmelia sinensis* and *Glycerine max* Extract; **HETCAM:** Hen's Egg Test Chorioallantoic Membrane; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **ROPT:** Repeated Opened Patch Test; **SCPT:** Single Closed Patch Test.

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10. Maily *et al.* Efficacy testing of anti aging complex 01 on healthy skin. Jakarta: Corporate Research PT. Martina Berto; 2016. Number of subject with positive response Table 2: Result of HETCAM Test on CCGEs: no significant difference to base was observed ($p > 0.05$)

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