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Original Article

THE FORMULATION OF NANOEMULSION SERUM-CONTAINING GOTU KOLA EXTRACT (*CENTELLA ASIATICA* L. URBAN) AND CHIA SEED OIL (*SALVIA HISPANICA*) FOR SKIN HYDRATION EFFECT

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ABSTRACT

Objective: The aims of this study of nano serum formulation from *C. asiatica* and chia seed to skin hydration effect.

Methods: *C. asiatica* extraction was macerated by 70% ethanol and evaporated by a rotary evaporator to produce high viscosity of extract yields. Four different formulas were obtained from nanoemulsion serum production with different concentrations of chia seed oil (1%, 2%, 3%, 4%). Two formulas F1 (1% chia seed oil) and F4 (4% chia seed oil) were selected for further clinical testing, which were applied at face skin for 7 d, then were measured using a skin analyzer looking for the best and most effective.

Results: Optimation result of extraction method of *C. asiatica* herb as high as 28.74% extract yields and 3.12% asiaticoside content. The result value of formulation based on the effect of moisturizing/hydration then can be analyzed with the Minitab program using the One-way ANOVA method. The result indicated that the product can increase face skin moistness at 20 respondents, where 13.36% increase from F1 formula and 13.88% increase from F4 formula.

Conclusion: The nanoemulsion serum-containing combination of *C. asiatica* extract and chia seed oil enables to increase in the moisturizing effect on dry skin or normal tend to dry skin.

Keywords: Nanoemulsion, *Centella asiatica*, asiaticoside, Chia seed, Hydration, Serum, Cosmetic

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INTRODUCTION

The human skin is an organ having the largest area in the human body. As located on the outer side of the human body, skin protects the human body from the outside environment [1]. The total area of skin in adults is approximately 1.5m^2 and it is about 15% of human weight. Various types of skin are normal, oily, and dry [1]. Moreover, there are various types of skin based on condition and characteristics; those are sensitive, dehydrated, aging, allergenic, moistness, and *oedematous* [1]. One of the problems of face skin is hydrated or moistness. This problem is caused by less water content in the skin. If skin moistness is low or water content is not adequate, the skin will dry. Water content in the stratum corneum (SC) in normal skin is approximately 10% in the outer layer and about 30% in the inner layer. If the water content in SC is less than 10% the skin will be scaly, rough, and dry. Moisturizer should be selected based on its capability to improve skin permeability [2].

Cosmetic containing synthetic chemicals can damage the skin because, naturally, humans have allergic reactions to chemical materials. Indonesia is known to have huge medicinal herbs, the second largest after Brazil. Medicinal plants in Asia, including Indonesia, are known to have good quality to be used as a natural cosmetic for skin and hair treatment. Natural plant resources currently become the most interesting topic for cosmetic product development, because it assumed to be safe and natural.

Moisturizer product content ingredients capable to moisturize are occlusive, humectant, emollient, and protein rejuvenator [2]. *C. asiatica* had functioned and was reported as a protein rejuvenator because containing some natural compounds such as asiaticoside that can enhance the AQP3 expression in NHEKs (Normal Human Epidermal Keratinocytes) [3]. The natural product having the characteristic as an emollient is Chia seed oil (*Salvia hispanica* L.) which contains omega 3 [4]. The researcher wants to combine *C. asiatica* extract and chia seed oil, to get a synergic effect for skin moistness.

C. asiatica is rich in amino acids, betacarotene, fatty acids, flavonoid, terpenoid, alkaloid, saponin, and several other nutrients [5]. *C. asiatica* extract is obtained by using 70% ethanol, resulting in the highest triterpene concentration compare to using other solvents by maceration [6, 7]. The higher the concentration of ethanol in extraction liquid, the higher asiaticoside concentration will be obtained. Chia (*Salvia hispanica* L.) is an edible seed from the yearly plant *Salvia hispanica* L. from the mint family *(Lamiaceae*). Containing good fatty acids omega-3, polyunsaturated fatty acids, fiber, and protein, including all essential amino acids, vitamins, calcium, and other important minerals [4]. Chia seeds also contain several phenolic compounds such as tocopherols, phytosterols, and carotenoids with their related antioxidant activity [8].

To combine water-soluble material and oil, a good formulation strategy is required to get a stable and well-blending mixture. Therefore, a good emulsifier is needed to obtain a good and stable emulsion product. For cosmetic products, besides their effect, the performance which attracts people is also necessary. Cosmetic product in the form of emulsion is less attractive. In this research, improvement of product performance, which is better than emulsion form will be studied. A soluble and transparent form of the product is the best choice. A transparent or translucent form of the emulsion can be produced by nanotechnology become nanoemulsions. Nanoemulsion is a transparent dispersion phase, thermodynamically stable with dropping size is about 5–200 nm. The stability between oil and water is supported by surfactant and co-surfactant. By this system, the target is to get the more stable product with extremely small globules in the nanosystem it will prevent creaming, sedimentation, and coalescent [9]. More than that, this product formulation will have high efficacy to penetrate stratum corneum at the skin [10, 11].

The present work attempted to formulate a nanoemulsion serum-containing combination of *C. asiatica* extract and chia seed oil enables to increase the moisturizing effect on dry skin or normal tend to dry skin.

MATERIALS AND METHODS

Materials

Materials used in this research are chia seed oil (Happy Green, Indonesia), glycerin (PandG Chemicals, USA), tween 80 (PT. Brataco, Indonesia), span 80 (PT. Brataco, Indonesia), propylene glycol (SK Picglobal, South Korea), PEG 40 HCO (Industria Chimica Panzeri S. r. l., Italy), butylene glycol (KH Neochem, Japan), phenoxyethanol, *fragrance* (Charabot, France), ethanol 96% pharma grade (PT. Brataco, Indonesia), distilled water (PT. Brataco, Indonesia), and dried leaves/of *C. asiatica* provided by Tawang Mangu Medicinal Plant Center, Central Java, Indonesia.

Pieces of equipment used in this research are analytical balance (RADWAG, Poland), grinder, 30 mesh sifter, incubator shaker (Innova 40, Germany), oven, furnace 1400, rotary evaporator (Heidolph, Germany), and (Buchi Rotavapor R-220, Switzerland), hotplate stirrer (Heidolph, Germany), hight speed homogenizer (Heidolph, Germany), ultrasonicator (Vibra-Cell, USA), pH meter (*Smart Sensor*, China), centrifugation (Thermo Scientific, USA), spectrophotometer UV-Vis (*Agilent Technologies Cary 8454 UV-Vis,* USA), Zetasizer ("Malvern Panalytical ", United Kingdom), skin tester (A one Tab, Korea), Karl-Fischer moisture titrator for water content, and glass equipment.

Production and evaluation method of *C. asiatica* herb

The evaluation process of *C. asiatica* herb starts from making Simplicia powder using a grinder, then sifting by mesh no.30. Simplicia standard evaluation based on Indonesia Herbal Pharmacopoeia: Simplicia identification, drying losses, total ash content, acid insoluble ash content, water-soluble extract, and ethanol-soluble extract [12].

Optimization of extraction method of *C. asiatica* herb

Optimization of *C. asiatica* extraction method with the variation of time and solvent ratio with 70% ethanol. To decide the right method to prepare *C. asiatica* extract, it is suggested to perform an optimation test of the solvent to be used. The optimation test was performed in two ways. The first test is to look for the optimum value of the amount of 70% ethanol solvent to the parameter of the percentage of extract yield. The second test is to look for the value of optimum maceration time to the percentage of extract yield.

Production and examination of high concentration of *C. asiatica* herb

After conducting an optimation test of comparison of solvent volume to maceration time of herb, maceration then was performed. This solidified extract then was calculated its extract yield and tested its moisture content, residual solvents and asiaticoside concentration obtained. Those results then were compared to the standard reference of Indonesian Herbal Pharmacopoeia [12].

Formulation and evaluation of nanoemulsion serum product

C. asiatica extract with 3.2% concentration is used in the formulation. The reference of concentration base of the journal from Linda Yulianti W, *et al.* 2017 [3] was mentioned that in 1 mg/ml asiaticoside can give the best result of hydrating/moisturizing. Chia seed oil in formulation performed with different concentrations (1%, 2%, 3%, and 4%), the concentration based on reference [13] that mentioned with 4% chia seed oil extract can give the best result of hydrating/moisturizing. Evaluation of nanoemulsion serum including organoleptic, homogeneity, pH, dispersion percentage, viscosity, distribution, adhesion, stability at room and 40 °C temperatures, centrifugation, stability at freeze and thawing, the particle size of nanoemulsion, irritation test, and hydration effect to skin of respondents. Ethical approval to conduct the study was granted by the Ethical committee KEPK UHAMKA Jakarta No 02/21.01/0820. The hydration/moistness effect was analyzed using an A-one Tab skin analyzer to measure the moistness value of water and oil content. Skin hydration/moistness indicates water content in the stratum corneum.

RESULTS AND DISCUSSION

Preparation of simplicia

The Preparation of simplicia was started to evaluate this powder according to the following parameters of Indonesian herbal Pharmacopeia [12]. The result is shown in table 1.

Table 1: Results of standardization of *C. asiatica* Herb

Parameters	Method	Value (%)	Reference limit (%)
Loss of Drying (LOD)	Moisture analyzer	8.3666±0.3055	<11
Total ash content	Gravimetry	11.8191±0.1135	<18.05
Acid insoluble ash content	Gravimetry	4.2635±0.2606	<4.9
Water soluble extract	Gravimetry	35.1933±0.0702	>28.3
Ethanol soluble extract	Gravimetry	8.9533±0.5832	>2.1

Note: Data were given in mean+SD, n = 3

Optimation of extraction method of *C. asiatica* herb result

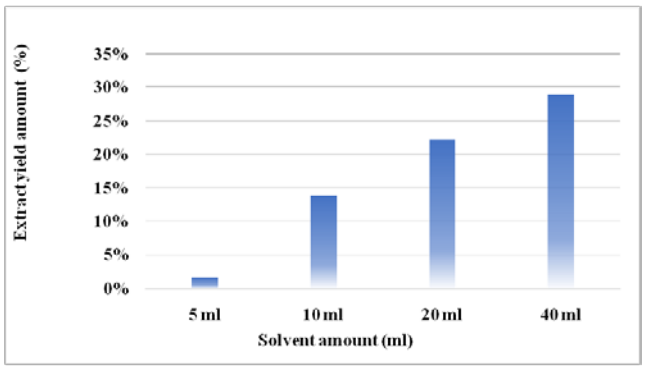

Fig. 1: Ratio between the amount of solvent (ml) 70 % ethanol and extract yield

From the above chart, it can be noticed the significant change of percentage of extract yield at the amount of solvent: 5 ml (2%), 10 ml (14%), and 20 ml (22%). At 40 ml solvent (29%), it was no significant change; therefore, it can be concluded that the optimum value that can be used is 20 ml (comparison between herb and solvent is 1:20).

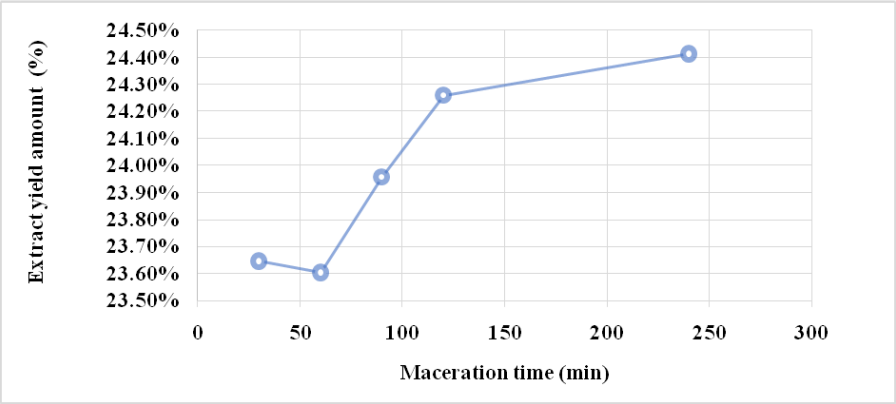

Fig. 2: Comparison between maceration time 1:20 to amount of extract yield

From the above chart, it can be seen significant change (sharp increase) at maceration time 90 min and 120 min and start to slow down at 240 min toward extract yield percentage. It can be concluded that the optimum peak is at 120 min with an extract yield is 24.26%.

The extraction process of *C. asiatica*

1 kilogram of *C. asiatica* leaves was macerated by 70% ethanol (1:20) for 2 h until dilute extract was obtained, and followed evaporated to obtain viscous extract using a rotary evaporator. This process was performed for 16 h at 50 °C until Continued to test evaluation (table 2).

Serum formulation and evaluation tests

After all components were prepared, formulas of nanoemulsion serum were started to be produced. All ingredients, including water phase, oil phase, and surfactant, were mixed using a magnetic stirrer, then followed by a homogenizer, and finally prepared into nanosize using ultrasonicator. The product then was packed into a small bottle equipped with the pipette; these four formulated serum then was ready to be further tested as follow.

Clinical test

As a good nanoemulsion serum, besides producing safe and stable products (as shown in table 4) it should also give benefit as moisturizing serum. Testing was conducted with 2 formulas which were F1 containing the lowest percentage of chia seed oil (1%) and formula 4, having the highest content of chia seed oil (4%). Moistness evaluation was performed into the face skin of 20 respondents, men and women at the age of 20-40 y old, using a skin analyzer portable A-One tab. The evaluation was performed before and after serum application once a day for 7 d. The result of the clinical test suggested that there is an increase in moistness/hydration of face skin, as shown in table 5.

Table 2: Result of *C. asiatica* viscous extract evaluation

Parameter test	Method	Value	Reference
Extract Yield Amount (%)		28.74	>7.2
Water Content (%)	Karl Fischer	7.23	<10
Ethanol solvent residue (mg/kg)	GC	Not Detected	2.8
Asiaticoside level (%)	TLC Scanner	3.12	>0.9

Table 3: Result test for organic chia seed oil (brand: Happy Green)

Parameter test	Method	Chia seed oil
Omega 3 (mg/100g)	GC	65455.8
Omega 6 (mg/100g)	GC	18290.6
Acid value	Titrimetry	5.80
Iodine value	Titrimetry	29.80
Saponification value	Titrimetry	200.32

Table 4: Test for nanoemulsion serum combination of *C. asiatica* extract and chia seed oil

Parameter test	F1	F2	F3	F4
Organoleptic	Liquid, slightly thick, transparent brown color, and smells of fragrance "macana"			
Particle size (d. nm)	29.08	22.58	21.23	23.43
Homogeneity	Homogen	Homogen	Homogen	Homogen
pH	5.74	5.75	5.75	5.73
Transmittan (%)	102.30	99.17	103.12	101.07
Spreadability test (cm)	12.6	12	11	9.2
Adhesive test (minutes)	>10	>10	>10	>10
Viscosity (cP)	51.3	64.0	90.0	116.7
Temperature stability	Stable	Stable	Stable	Stable
Mold and yeast contamination (colony/**ml**)	<10	<10	<10	<10
Microbial contamination (ALT) (colony/**ml**)	4.1×10^2	4.8×10^2	4.3×10^2	4.5×10^2
Irritation test	There are no signs of irritation			
Centrifugation test	No precipitation or phase separation was found			
freeze-thaw test	Color and viscosity Stable and there is no phase separation			

Table 5: Moisture test results data on 20 respondents

No	Sex	Age (y. o.)	Moisture value before applying serum		Moisture value after seven days of applying serum	
			Right cheek apply F1	Left cheek apply F4	Right cheek apply F1	Left cheek apply F4
1	Woman	23	33 (L)	31 (L)	39 (N)	36 (L)
2	Woman	21	34 (L)	36 (L)	39 (N)	37 (N)
3	Woman	25	33 (L)	32 (L)	36 (L)	37 (N)
4	Man	20	29 (L)	30 (L)	34 (L)	32 (L)
5	Woman	34	31 (L)	26 (L)	39 (N)	38 (N)
6	Woman	40	33 (N)	36 (N)	36 (N)	36 (N)
7	Man	20	33 (L)	40 (N)	33 (L)	40 (N)
8	Woman	40	33 (N)	27 (L)	33 (N)	30 (L)
9	Woman	25	33 (L)	33 (L)	40 (N)	40 (N)
10	Woman	40	36 (N)	38 (N)	40 (N)	39 (N)
11	Woman	40	34 (N)	34 (N)	34 (N)	34 (N)
12	Woman	40	35 (N)	35 (N)	36 (N)	37 (N)
13	Woman	36	30 (L)	32 (L)	36 (N)	36 (N)
14	Woman	34	33 (L)	33 (L)	35 (N)	35 (N)
15	Woman	23	27 (L)	33 (L)	38 (N)	36 (L)
16	Woman	31	30 (L)	32 (L)	34 (N)	37 (N)
17	Woman	40	32 (N)	34 (N)	34 (N)	34 (N)
18	Woman	20	33 (L)	32 (L)	38 (N)	42 (N)
19	Woman	20	23 (VL)	15 (VL)	34 (L)	33 (L)
20	Woman	40	31 (N)	25 (N)	33 (N)	33 (N)

Description: Moisture value is the value that is read on the tool or the test equipment, description of water content on facial skin (VL= very low, L= low, N=Normal)

Statistical analysis with One-way ANOVA method with Minitab 19 software indicated that application of formula 1 (1% Chia seed oil) and formula 4 (4% Chia seed oil) after 7 d compare to before application shows the result as follow:

Table 6: Analysis result for comparison before applying serum (F1 and F4) and after seven days of applying

Formula	p-value	Tukey method	
		before	after 7 d
F1	0.000*	31.800	36.050
F4	0.003*	31.700	36.100

*P<0.05, Tukey Method based on Mean grouping

Based on the result of statistical analysis of One-way ANOVA, the p-value of formula 1 (F1) is 0.000 (*P<0.05), where 0 Hypotheses are rejected, meaning there are significant improvements of moistness after using formula 1 for 7 d. Further test with Tukey test, it is shown that after application formula 1 for 7 d, the average value is 36.050, an increase 13.36%, compared to that before application with value is 31.800.

Based on the result of statistical analysis of One-way ANOVA, the p-value of formula 4 (F4) is 0.003 (*P<0.05), where 0 hypotheses are rejected, meaning there is a significant improvement of moistness after using formula 4 for 7 d. Further test with Tukey test, it is shown that after application formula 4 for 7 d, the average value is 36.100, an increase 13.88%, compared to that before application with value is 31.700.

Table 7: Analysis result of improvement moisture content after applying serum F1 and F4

Performance	p-value	Tukey method	
		F0-F1	F0-F4
Improvement moisture content after use	0.907	4.250	4.40

*P<0.05, Tukey Method based on Mean grouping

Based on the result from the One-way ANOVA statistical analysis, the average value of skin moistness of F1 and F4, shows that moisture content improvement from F4 (4.40) is higher than moisture content improvement from F1 (4.250), the p-value is 0.907 (*P>0.05) where 0 Hypothesis are accepted, meaning there is no significant difference between performance after use F1 and F4.

CONCLUSION

The optimal ratio of 1:20 and a maceration time of 2 h resulted in an extract yield of 28.74% (above the allowable reference value>7.2%). Production of *C. asiatica* herb extract, standard results were obtained, both simplicia and viscous extract which were following the standard value of the herbal pharmacopeia where the value of asiaticoside content was 3.12% (above the reference value of the Indonesian Herbal Pharmacopoeia, which was>0.9%). In this study, the results of the nanoemulsion formula were obtained which have a transparent, stable appearance, easy to apply to facial skin, and do not irritate during testing. The nanoemulsion preparations produced in this study had good storage stability. The nanoemulsion preparations produced in this study have been tested on respondents and have a good moisturizing effect; based on statistical analysis, there is a significant difference from before and after the use of serum for 7 d where the best formula is with the concentration of 4% chia seed oil (F4), with an increase in the value of hydrating of 13.88%.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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