

Effectiveness Test of Centella Asiatica Extract on Improvement of Collagen and Hydration in Female White Rat (*Rattus Norwegicus Wistar*)

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Abstract

Gotu Cola (*Centella asiatica*) is one of the plants used as a skin care product because it contains triterpenoid, asiaticosida, madekakosida, flavonoids, which have antioxidant, anti-inflammatory properties and prevent premature aging. The purpose of this study was to compare the effectiveness of *Centella asiatica* extracts against increased levels of collagen and hydration in the skin of female white rats. This study is a non-experimental and experimental study, using a pre-test design post-test control group design. Non-experimental studies include the determination of gotu kola plants, extraction and manufacture of anti-aging cream preparations using gotu kola extract with concentrations of 2.5%, 5%, 7.5%, and 10%. Experimental studies include testing anti-aging activities. Data were analyzed by linear regression analysis. The results showed that the cream extract of *C. asiatica* 10% showed the highest increase in collagen levels by an average of 77.89% and an average hydration of 81.58%. Cream *Centella asiatica* 2.5% cream showed the lowest increase in collagen levels by an average of 21.12% and an average hydration of 39.40%. Concentration and time had a positive effect on collagen levels and hydration of female white rats. Conclusion: The administration of gotu kola herb extract cream increased the average collagen levels and skin hydration compared with controls. *C. asiatica* extract can be used as an alternative anti-aging agent in the science of dermato-cosmetology. Further research needs to be done using different measuring devices and also different parameters.

Keywords: anti aging; *Centella asiatica*; collagen; hydration.

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1. Introduction

The skin is the biggest organ and serves to protect the body from the external environment such as shock, temperature, ultraviolet radiation, chemical and other threat. The skin consists of three layers, namely epidermis which functions as a liquid binder, a protector from foreign invasion, as a sensory organ, giving color to the skin and protecting internal organ, then the dermis layer that functions as regulating body temperature, providing moisture to the skin, and as a sensory place on the skin, and then subcutaneous fat tissue that functions as a protector from heat and cold and as a center for energy storage [1:50-54]. Change in the skin can be recognized as a visual information and is associated with the aging process. The aging factor from the perspective of beauty is a decrease in skin texture, appearance of fine line and wrinkle, decrease elasticity and resilience, loss of skin texture that is elastic and supple and decreased skin function.¹ The aging process in human can be divided into 2 factors, namely intrinsic factor consisting of age, sex, hormone, and immune process as well as extrinsic factor such as trauma, free radical, and UV ray [1:50-54]. The sun's ultraviolet ray is the main extrinsic factor causing skin aging or photoaging, through the formation of free radical. The skin that undergoes photoaging will experience an increase in inflammatory mediator, increase collagen degradation, decrease collagen synthesis, and increase elastotic tissue which will cause effect in the form of wrinkle, reduce collagen synthesis and reduce skin elasticity. Antiaging agent that is often used to reduce the effect of free radical is antioxidant that can be obtained through the outside of the body (exogenous antioxidant), one of which is through plants [2:1-17]. Skin care product made from herb has increased demand in the world market and is an invaluable gift of nature. Herbal formulation always attracts attention because of good activity and side effect that is relatively less or nothing with synthetic drug. Skin care product made from herb is defined as beauty product that has desired physiological activities such as healing, smoothing, improving skin condition. But, the use of synthetic product has been very dangerous for a long time for young people and our environment. Various synthetic compound, chemical, dye and their derivative are proven to cause various skin diseases that have many side effects. So, the solution is to use herbal skin care product as much as possible. A good skin care product must have varieties of properties such as antioxidant, anti-inflammatory, antiseptic, emollient, anti-seborrheic, antikerolytic and antibacterial activity. Herbal skin care product developed to reduce wrinkle, fight acne and control excess oil [3:1495-1497]. One of the herb that can be used as a skin care product now is gotu kola herb as well as being an herb that has antioxidant, gotu kola is also a plant that is easy to find and has various benefits such as anti-inflammatory and detoxification [2:1-17]. Gotu kola itself has different names depend on the place of origin. In West Java, it is called *antan*. In Jakarta and Aceh, the name of it is *gotu kola*, Sumatran people call it as a *horse leg plant*, There are many more local gotu kola names, such as *kori-kori* in Halmahera, *pegago* in the Minangkabau area, *dogauke* or *sandan* or *gogauke* in the Papua region, and *bebile* in the Lombok area . The name of gotu kola in several countries include *gotu kola* in Sri Lanka, *takip-kohot* in the Philippines, *penny wort* in England, *brahma butu* in India, *penny wort* in England and in China known as *ji xue cao*, while in France it is known by the name *bevilaque*, *hydrocote d'Asie*, or *cotyiole asiatique* [4:121-130]. Gotu kola plant (*Centella asiatica*) contains active compound flavonoid and triterpenoid that function as antioxidant [5:1-9]. The effect is associated with increased collagen content on the skin [6:46-49]. Collagen level is the most commonly used anti-aging parameter. In addition, anti-aging parameter can also use sensitivity, moisture content, elasticity, and pore size [7:1-7]. In this study, the effectiveness of gotu kola extract will be tested on female white mice

because it is suspected that there is an anti aging effect of gotu kola and hormonal effect that can strengthen the anti aging effect.

2. Material and Method

2.1. Research location

The study was conducted from February to April 2019 at the Center for Animal Development for North Sumatra Research.

2.2. Experimental design

The type of research is a non-experimental and experimental research, using a pre-test design post test control group design. Non-experimental studies include the determination of gotu kola plant, extraction and manufacture of anti-aging cream preparation using gotu kola extract with concentration of 2.5%, 5%, 7.5%, and 10%. Experimental research includes testing anti aging activity. Anti aging activity testing was carried out on 25 white rats with 6-8 weeks of age and weight 150-200 mg.

2.3. Implementation method

Making gotu kola extra: Gotu kola plants from Purbalingga are dried and then made into powder using a blender. Gotu kola plant powder (*Centella asiatica*) weighed 100 grams then macerated with 400 ml of 70% ethanol for 24 hours with stirring. After maceration treatment the solution is then filtered using vacuum and filter paper to separate the pulp and the filtrate, the filtrate is then separated to evaporate while the pulp is recovered with a new solvent. This treatment is carried out three times or when the liquid extract is clear. The filtrate is then evaporated with a rotary evaporator at 60°C, so that a thick extract is obtained from gotu kola plants. Phytochemical examination: alkaloid examination, tannin examination, saponin examination, triterphenoid examination, flavonoid examination, glycoside examination. Cream formulation: Cream preparation is made based on a standard formula that uses a basic type of oil in water cream:

Table 1: Cream Formulation [8:10-11]

Composition	Concentration				
	P0	P1	P2	P3	P4
C. asiatica extract	-	2,5%	5%	7,5%	10%
Stearat acid	7,5	7,5	7,5	7,5	7,5
Triethanolamine	0,75	0,75	0,75	0,75	0,75
Natrium benzoat	0,1	0,1	0,1	0,1	0,1
Gliserin	5	5	5	5	5
Aquadest	Ad	Ad	Ad	Ad	Ad
	50	50	50	50	50

Gotu kola extract concentrations used in the manufacture of anti-aging cream preparation are 2.5%, 5%, 7.5%,

and 10% of the basic formulation of the cream without gotu kola extract is made as a placebo. Cream preparation: Stearic acid oil phase and aqueous phase (triethanolamine, glycerin, sodium benzoate and distilled water) each melted in a porcelain cup over a temperature of 70°C. The oil phase is poured into the water phase, stirring in warm to cold mortars. The ingredients for each treatment are poured into another mortar, then added a cream base little by little, while stirring until homogeneous. Testing anti-aging activity: Testing anti-aging activity using 25 samples of female mice and divided into 5 groups:

Group 1 (KN): 5 female rats for cream A (Blank)

Group 2 (P1): 5 female rats for cream B (Extract 2.5%)

Group 3 (P2): 5 female rats for cream C (Extract 5%)

Group 4 (P3): 5 female rats for cream D (Extract 7.5%)

Group 5 (P4): 5 female rats for cream E (Extract 10%)

The entire group of rats are previously dried in the sun for 5 days for 4 hours (9:00 to 13:00), then, the fur is shaved on the back area of 2x2 cm² using an electric shaver and a manual hair shaver. Then, measure the condition of the test animals before treatment according to research conducted by Surjanto with a Skin Analyzer EH 900 U including water content and collagen content. After measuring the initial skin condition, treatment is started by applying a thin and evenly applied cream to an area that has been marked, applying cream based on the group specified above, applying it twice a day for 4 weeks. Changes in skin condition are measured every 1 week for 4 weeks using a skin analyzer [9:99-104].

2.4. Data analysis

The anti aging activity data of Centella asiatica extract is analyzed by the SPSS 25 program. The data are tested for normality by the Shapiro-Wilk test and homogeneity is tested by the Levene's test. If the data is normal and homogeneous, the data is then tested with the Repeated Anova Test, followed by the Pearson correlation test and Multiple Linear Regression. If the data are not normal, use the Kruskal-Wallis test analysis followed by the Mann-Whitney test and the Multiple Linear Regression Test.

3. Result

3.1. Description of phytochemical test result

Phytochemical test result on Centella asiatica extract is presented in Table 2.

Table 2: Phytochemical test results of *Centella asiatica* extract

No.	Parameter	Result	Sign
1	Alkaloid	Positive	+
2	Tanin	Positive	+
3	Saponin	Positive	+
4	Triterpen/Steroid	Positive	+
5	Flavonoid	Positive	+
6	Glikosida	Positive	+

3.2. Collagen

The result of examination of collagen level in the skin of female white rats smeared with *Centella asiatica* extract cream is shown in Table 3.

Table 3: Collagen levels of female white rat skin after being centrelated with *Centella asiatica* extract cream

Collagen						
Formula	Before	Week 1	Week 2	Week 3	Week 4	% Collagen increasement
P0 (kontrol)	38,4	39,2	40	40,6	41,2	7,2
P1 (2,5%)	39,8	41,8	44,4	46,2	48,2	21,12
P2 (5%)	38,4	42	45,4	49,6	55	43,2
P3 (7,5%)	39,6	45,8	51,2	56,2	63,2	57,54
P4 (10%)	39	48,8	53,4	60,8	68,8	77,89

Explanation:

Normal 25-50%; Good 50-65%; Very Good 65-80%

P : Formula

P0 : Control

P1 : Gotu kola herb extract cream 2.5%

P2 : Gotu kola herbal extract 5%

P3 : Gotu kola herb extract 7.5%

P4 : Gotu kola herb extract 10%

Kadar Collagen level in all groups of rats before applying gotu kola herb extract cream is 35-42 (normal). After rubbing with gotu kola herb extract cream and with gotu kola herb extract for four weeks, cream with gotu kola extract or cream without extract of gotu kola herb showed an increase in collagen level. The application of cream with or without Centella asiatica extract for 4 weeks together increases the collagen levels of the skin of female white rats. However, cream with Centella asiatica extract 10% shows the highest increase in collagen level by an average of 77.89% with an average final value of 68.8 (very good). Whereas, cream with Centella asiatica 2.5% extract shows the lowest increase in collagen level by an average of 21.12% with an average final value of 48.2 (good), when compared to other Centella asiatica extract cream (5%, 7.5%, 10%). Whereas, the cream without Centella asiatica shows an increase in collagen level by an average of 7.2% with an average final score of 41.2 (normal) for four weeks of treatment.

3.3. *Hidration*

Result of examination of hydration level in the skin of female white rats smeared with Centella asiatica extract cream is shown in Table 4.

Table 4: Hydration levels (water) of female white rat skin after being applied with Centella asiatica extract cream

Hydration							
Formula	Before	Week 1	Week 2	Week 3	Week 4	%	Hydration increasement
P0 (Kontrol)	27,4	29,6	31,6	32,6	34,2	25,18	
P1 (2,5%)	27,6	30,2	32,2	35,2	37,4	39,4	
P2 (5%)	26	29,4	33	37	42	64,03	
P3 (7,5%)	28,2	33,4	39,8	45,6	50	74,71	
P4 (10%)	27,8	34,2	40	45,8	51,6	81,58	

Explanation:

Dry: 3-4%; Less: 4-10%; Normal: 10-15%; Height: 15-30%; Very high:> 30%

P : Formula

P0 : Control

P1 : Gotu kola herb extract cream 2.5%

P2 : Gotu kola herbal extract 5%

P3 : Gotu kola herb extract 7.5%

P4 : Gotu kola herb extract 10%

Hydration level in all groups of female white rats before applying the gotu kola herb extract cream is 22-31 (high). After applying with Centella asiatica herbal extract cream or with cream without Centella asiatica extract for four weeks, cream with extract or cream without Centella asiatica extract shows an increase in hydration levels. The application of cream with or without Centella asiatica extract both increases hydration level during the four weeks of treatment. However, cream with Centella asiatica 10% extract shows the highest increase in hydration level by an average of 81.58% with an average final value of 51.6 (very high). Whereas, cream with Centella asiatica 2.5% extract shows the lowest increase in elasticity level by an average of 39.40% with an average final value of 37.4 (very high), when compared to other Centella asiatica extract cream (5%, 7.5% , 10%). Whereas, the cream without Centella asiatica shows an increase in hydration level by an average of 25.18% with an average final score of 34.2 (high) for four weeks of treatment.

4. Discussion

4.1. *The Effect of Centella asiatica extract cream on collagen level*

Application of cream with or without Centella asiatica extract for 4 weeks together increases the collagen level of the skin of female white rats. The result of this study is consistent with research conducted by Lee and his colleagues [10:324-329] who observed that Centella asiatica preparation can stimulate fibroblast proliferation and activate SMAD signaling pathways so as to increase type I collagen production and reduce the formation of stretch marks and inflammatory reaction. In addition, the constituent isolated from *C. asiatica* can improve blood circulation in the skin and prevent excessive accumulation of fat in cell. Binding induced asiaticoside from SMAD 3 and SMAD 4. In consistent use, complex nuclear translocation of SMAD 3 and SMAD 4 is induced through treatment with asiaticoside, indicating there is involvement of asiaticoside in Smad signaling. In addition a TGFbeta receptor I (TbetaRI) kinase inhibitor, known as an SMAD pathway activator, is not found to inhibit Smad 2 phosphorylation synthesis and Type 1 collagen synthesis induced by asiaticoside. On this study shows that asiaticoside in *C. asiatica* can induce synthesis of type I collagen through activation of the TbetaRI kinase-independent SMAD pathway. As for cosmetic purposes, Centella asiatica is used as an active compound in skin care preparation because of its antioxidant, anti-inflammatory, anti-cellulite, and antiaging activities. Therefore, Centella asiatica extract which is rich in triterpenes is a valuable raw material with a broad spectrum of cosmetic action. The result of a study conducted by Hashim Lee and his colleagues [11:1310-1322] who looked at the effects of the triterphenoid composition and bioactivity of *C. asiatica* compared with Vitamin C showed that the extract of *C. asiatica* showed a stimulatory effect on collagen synthesis in doses that were dependent on the treatment method. Data are expressed as μg collagen synthesized per 100 μg of total cellular protein to standardize the total number of cell from each plate. Vitamin C (25 μg / mL) used as a positive control showed a 2-fold collagen enhancer response. At 50 mg / mL Centella extract increased collagen production 3-fold compared to the (untreated) control, whereas at 30 mg / mL and 10 mg / mL collagen increased 2 and 1.4 times respectively. Centella extract at concentrations higher than 50 mg / mL, cell viability decreases (data not shown). The result obtained is in accordance with previous studies in which triterpenes

extracted from *Centella* stimulate collagen synthesis. In the cosmetics world, *C. asiatica* has been used as an effective anti-photoaging agent, mainly due to an increase in type I collagen, which amounts for aging decreases with age. This action was confirmed in a double randomized clinical trial conducted by Haftek Lee and his colleagues [12:946-952] among 20 female participants (45-60 years old) with skin photaging to examine the impact of 0.1% madecassoside applied topically in mixed with 5% vitamin C on their skin. Six-month treatment resulted in a significant increase in skin firmness, elasticity and hydration, as well as the level of collagen that was confirmed by biometry tests. It is thought that the beneficial effect of *C. asiatica* on improving skin condition is due to madecassoside, an induction of collagen expression that is known by activating the SMAD signaling pathway. In previous studies, the same researchers confirmed the beneficial effect of 5% vitamin C on photoaging skin, which results from stimulation of collagen synthesis in fibroblasts and matrix metalloproteinase control which is responsible for collagen degradation, whereas in skin with photoaging, the level of vitamin C in tissue significantly reduced. Thus, it can be concluded that a mixture of vitamin C and madecassoside is an interesting combination of two active compounds that are characterized by a mechanism different activities, which provide an additive or synergistic effect. In research conducted by Farage, Miller, Elsner, & Maibach [13:87-95] shows that Collagen is influenced by extrinsic and intrinsic factors. Intrinsic factor includes genetic factor and hormone such as estrogen, extrinsic factor includes ultraviolet light, pollution, and diet. Estrogen can increase collagen synthesis. Menopausal women experience a significant decrease in collagen level. In human, estrogen is also associated with an overall increase in the deposition of collagen during the remodeling phase. This suggest estrogen can affect the balance between collagen synthesis and degradation and research has shown that although aging is associated with increased expression of Matrix Metallo Proteinase (MMP), specifically MMP-2 and MMP-9, staining for MMP-9 is most evident in older women, show that estrogen reduction that occurs postmenopausal can affect proteinase production [14:264-270].

4.2. *The Effect of Centella asiatica cream extract on hydration level*

The application of cream with or without *Centella asiatica* extract both increases hydration level during the four weeks of treatment. The result of this study is consistent with research conducted by Saraf, Chhabra, Kaur, & Saraf (2012) which shows that there are increased levels of hydration, sebum, viscoelasticity and decreased levels of melanin in the use of herbal creams with the basic ingredients of *C. asiatica* (1-5% w/w) caused by the effects of active constituents (polyphenols and glycosides) contained in hydroethanolic extract from selected herbs that function as antioxidant, anti-elastase, antiproliferative, photochemoprotective, antipyretic, anti-allergic, astringent, facemask-toning, and anti-aging. Hydroalcoholic extract from *C. asiatica* is used to make herbal cream along with four medicinal plant (*Curcuma caesia*, *Areca catechu*, *Cinnamon zeylanicum* and *Tamarindus indica*) [15:119-131]. Another study is comparing the administration of *Centella asiatica* with the Microneedle Therapy System (MTS) method on the right face (experimental group) and the administration of hyaluronic acid as a control group on the left face (control group) in 14 women for 8 weeks on increasing level of hydration, shows that a significant increase in skin hydration level is found in both the experimental and control groups ($p < .0001$) [16:787-794]. In a study conducted by Milani, Sparavigna, Cooper, Pertusella, & Varese (2017) who looked at the comparison of skin barrier function and skin hydration for 24 hours on moisturizers with 1% hyaluronic, Glycerin 5% and *C. asiatica* extract with the control group found that there is

an increase in skin hydration (+ 59% after 1 hour, + 48% after 8 hours, and + 29% after 24 hours) compared with the control group. This is because HA and glycerin are substances that are believed to increase skin hydration because they play a role in skin reepitalization and increase the proliferation and activity of keratinocytes and *C. asiatica* extract is rich in amino acid, flavonoid, terpenoid, essential oil and alkaloid. *C. asiatica* extract can inhibit the activity of the hyaluronidase enzyme and prolong the effect of HA [17:311-315]. This is consistent with research conducted by Nema, Maity, Sarkar, & Mukherjee (2013) who see an inhibitory effect of hyaluronidase, metalloproteinase and elastase on *C. asiatica* extract by assessing enzymatic activity with ursolic acid and oleanolic acid. As a result, *C. asiatica* extract has significant inhibitory activity namely $IC_{50} = 27.00 \pm 0.43$ and $18.63 \pm 0.33 \mu\text{g/mL}$ in hyaluronidase and elastase $IC_{50} = 29.15 \pm 0.31$ and $19.45 \pm 0.25 \mu\text{g/mL}$. This proves that the extract *C. asiatica* can be used as an anti-aging agent [18:1182-1187].

5. Conclusion

Cream of *C. asiatica* extract 10% shows the highest increase in collagen level by an average of 77.89% and an average hydration of 81.58%. Cream *Centella asiatica* 2.5% cream showed the lowest increase in collagen level by an average of 21.12% and an average hydration of 39.40%. Conclusion: The administration of gotu kola herb extract cream increased the average collagen level and skin hydration compared to control, positively correlated with the increase in duration of gotu kola extract cream and the amount of gotu kola extract given. Gotu kola extract can be used as an alternative anti aging agent in the science of dermato-cosmetology. Further research needs to be done using different measuring devices and also different parameters.

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