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Comparison of Anti-Aging Effectiveness from Gotu Kola Extract Cream (*Centella asiatica)* and Robusta Coffee Cream *(Coffea canephora)* Toward Hydration Levels in Male Mus Musculus Skin

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Abstract

Gotu Kola (Centella Asiatica) and Robusta coffee (Coffea Canephora) are natural ingredients that are easily found in the Indonesia environment and contain various types of antioxidants that can provide anti-aging effects or prevent premature aging so that the potential is large enough for development in the science of dermatocosmetology. The aim of this research is to compare the effectiveness of anti-aging extracts of gotu kola herb and robusta coffee toward the levels of hydration in the skin of male white mice. The research data that were processed showed that the data were abnormally distributed ($p < 0.05$). The highest percentage increase in skin hydration level was found in the 10% Robusta coffee treatment group followed by 10% gotu kola herb extract and control. The mean percentage increase in skin hydration levels in all treatment groups was 43.6% with a standard deviation of 27.3. The percentage difference of hydration levels in skin among these groups was significant ($p < 0.005$).

Keywords: Centella asiatica; Robusta coffee; anti-aging; hydration.

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1. Introduction

As an organ that is located in the outermost layer of the body, the skin also functions as a barrier to protect the body against environmental influences such as temperature, ultraviolet rays, radiation, chemicals, pollution, and other threatening materials that will affect skin aging. Besides that, there are also intrinsic factors such as phenotypic changes in skin cells as well as the structural and functional changes in the extracellular matrix components such as elastin, collagen, and proteoglycans which is to provide elasticity, tensile strength, and hydration to the skin [1, 2, 3]. ROS (Reactive Oxygen Species) that induced by UV rays, air pollution, or other extrinsic exposure to the skin can increase oxidative stress, when their formation exceeds the antioxidant defense capabilities of the target cell, resulting in an imbalance between oxidants and antioxidants [4]. The application of anti-aging creams to the skin can brighten skin tone, moisturize the skin, and make a younger appearance. However, most synthetic anti-aging creams can cause some side effects such as allergic reactions so that anti-aging creams from herbal plants can be used safely on the skin to prevent side effects and allergic reactions [5]. In these recent years there have been many researches focused on the natural ingredients that are around us, including research in the beauty industry. Gotu kola (Centella asiatica) and robusta coffee (Coffea canephora) is the one of many natural ingredients that can be considered to be used. The asiaticoside, madecassoside, asiatic acid, and madecasat acids contained in Centella asiatica have anti-oxidant activity that can increase skin moisture [6, 7]. Polyphenols, alkaloids, tannins, and saponins that contains in Coffea canephora has quite high antioxidant activity. Where polyphenols are most contained in coffee are chlorogenic acid and caffeic acid [8, 9, 10]. The purpose of this research is to compared the effectiveness From Gotu Kola Extract Cream (*Centella asiatica)* and Robusta Coffee Cream *(Coffea canephora)* Toward Hydration Levels in Male Mus Musculus Skin

2. Material and Methods

2.1. Materials

The materials used are distilled water, glacial acetic acid, 2N hydrochloric acid, 2N sulfuric acid, concentrated sulfuric acid, ethanol 96%, FeCl3 10%, isopropanol, Bouchardic reagent, Dragendorff reagent, Meyer reagent, Molisch reagent, lead ethanol (II) acetate, gotu kola, and robusta coffee.

2.2. Instruments

Drying cabinets, trays, blenders, sieves, glassware, analytical scales, maceration vessels, filter paper, rotary evaporators, waterbaths, glassware, analytical scales, porcelain cups, mortars, stamper, spatula, and ointment pots.

2.3. Extract Manufacture

The plant material used are Centella asiatica herbs. Centella asiatica herbs washed clean and dried in a drying cupboard for 3 days (72 hours) with a temperature of ± 40^{o}C to dry which is marked by simplicia easily broken [11]. The simplicia is weighed and blended to simplicia powder and then put in a plastic bags, given etiquette and stored in a dry place [12]. 10 parts of gotu kola simplicia powder (1 kg of simplicia powder) were immersed

in 75 parts of 70% ethanol (7.5 liter) liquid in a maceration vessel and then covered, left for 5 days with shielded from sunlight while stirring daily. The maserate is filtered, then the filtrate is macerated again with 25 parts of 70% ethanol (2.5 liter) liquid in a in a closed container, left in a cool place and sheltered from sunlight for 2 days, then filtered. The maserate obtained was concentrated by concentrating with a rotary evaporator at 400-500C until a thick extract was obtained. The resulting thick extract is left at room temperature until all the ethanol solvents evaporate. The extract was weighed and stored in a closed glass container before being used for testing [13]. Coffee bean extract is made using robusta coffee beans (Coffea cenephora) which have been ground into powder. Making robusta coffee bean extract is done using maceration techniques. A total of 120 g of coffee powder powder was put into the erlenmeyer, then soaked with a 96 mL ethanol solution of 225 mL, covered with aluminum foil and left for 5 days while occasionally stirring. After 5 days, the samples soaked were filtered using filter paper to produce filtrate 1 and pulp 1. The remaining pulp was then macerated with 96% ethanol solution as much as 75 mL, covered with aluminum foil and left for 2 days while occasionally stirring. After 2 days, the sample is filtered using filter paper to produce filtrate 2 and pulp 2. Filtrate 1 and 2 are combined, then evaporated using a rotary evaporator, so that Robusta coffee bean extract is obtained. The thick extract is left at room temperature until all the ethanol solvents evaporate. The extract was weighed and stored in a closed glass container before being used for testing [14].

2.4. Phytochemical Methods

- ***Alkaloid Test***

Weighed each simplicia and extract as much as ± 0.5 g, then added 9 ml of distilled water and 1 ml of 2-N-hydrochloric acid, heated on a water bath for 2 minutes, then chilled and filtered. The resulting filtrate obtained is used for alkaloid test. 3 test tubes were taken, then 0.5 ml of filtrate were added. To each test tube 2 drops of major reagent, bouchardate reagent, and dragendorff reagent were added. Alkaloids are positive if there is sedimentation or turbidity in two of the three experiments above [15].

- ***Tannins Test***

Weighed each simplicia and extract as much as ± 0.5 g, mixed with 10ml of aquadest and filtered, the resulting filtrate was diluted with aquadest until it was colorless. The resulting solution is taken as much as 2 ml and added 1-2 drops of reagent iron (III) chloride 1%. If there was blue or blackish green color that showed the presence of tannins [15].

- ***Saponin Test***

Weighed each simplicia and extract as much as ± 0.5 g, and put into a test tube, then added 10 ml of hot water, chilled, then shaken hard for 10 minutes. If the foam is formed as high as 1-10 cm stable not less than 10 minutes and the froth is not lost with the addition of 1 drop of 2-N-hydrochloric acid indicating the presence of saponins [15].

- ***Flavonoid Test***

Weighed each simplicia and extract as much as ± 0.5 g and added 20 ml of hot water, boiled for 10 minutes and filtered under heat, added 0.1 g of magnesium powder, 133 ml of concentrated hydrochloric acid, and 2 ml of amyl alcohol in to 5 ml of resulting filtrate, then shake and allowed to separate. Flavonoid is positive if there is red, orange, and yellow color in the amyl alcohol layer [15].

- ***Triterpenoid Test***

Weighed each simplicia and extract as much as ± 1 g, macerated with 20 ml N Hexane for 2 hours, and then filtered. The resulting filtrate was evaporated in a vaporizer cup and then Liebermann-Burchard reagent is added through the cup wall. If a red or purple color changes to blue purple or blue green indicates the presence of triterpenoids / steroids [15].

- ***Glycosides Test***

Each extract weighed ± 3 g extracted with 30 ml of distilled water mixture with technical ethanol in a ratio of 3:7, then refluxed for 10 minutes, chilled and filtered. Add 25 ml of distilled water and 25 ml of lead (II) acetate 0.4 M into 20 ml of the filtrate that was produced earlier, then shake and let stand for 5 minutes and then filtered. The resulting filtrate was extracted with 20 ml of a mixture of isopropanolol and chloroform (2:3), repeated 3 times. Evaporate the collection of water at temperature of not more than 50^{o}C. Dissolve the remainder in 2 ml of methanol. For experiments use the remaining solution which is put in a test tube and evaporated on a water bath. To the rest add 5 drops of molish reagent and 2 ml of water. Then slowly add 2 ml of concentrated sulfuric acid through the tube wall, if a purple ring is formed at the boundary of the two liquids, indicating there is a glucose bond [15].

2.5. Creams Formulation

Cream preparations are made according to a standard formula that uses a basic type of oil-in-water cream [16].

Table 1: Creams Formulation

Ingredients	Concentration (%)		
	F0	**F1**	**F2**
Gotu Kola extract	-	10	-
Robusta Coffee extract	-	-	10
Stearic acid	15	15	15
Setil alcohol	10	10	10
Vaselin	10	10	10
Mineral oil	12	12	12
Isopropyl palmitate	12	12	12
Glycerin	5	5	5
Triethanolamine	1	1	1
Fragrance	q.s	q.s	q.s
Preservative	q.s	q.s	q.s
Water	ad 100	ad 100	ad 100

2.6. Animal Trial Procedure

Anti-aging activity test was using 27 samples of male mice and divided into 3 groups. Group 1 treated by using F0 (control), group 2 treated by using F1 (10% Centella asiatica extract), and group 3 treated by using F2 (10% Coffea canephora extract). Mice are placed in a cage with a size of 70 cm x 50 cm x 17 cm, with a temperature of 25-27^{o}C, based on wood husk which is replaced every two days, light comes from the window during the day, cross ventilation on the top of the cage. 1 cage filled with 9 mice. All mice were acclimatized for 7 days with the aim that the test animals were able to adapt to the environment. The entire group of mice will be shaved carefully and slowly on the back of the back area of 2x2cm^2 using a manual shaver. Then measuring the hydration level of the animals before treatment with skin analyzer EH 900 U. After measuring the initial skin condition, treatment is started by applying the cream to evenly as wide as the area that has been marked, the cream is applied according to the groups specified above, the application is done twice a day for 4 weeks. Changes in skin condition were measured every week for 4 weeks using skin analyzer EH 900 U.

2.7. Statistical analysis

All data obtained were analyzed with the SPSS 25 program. All results were presented as mean±standart deviation for each concentration of sample. Statistical analysis was performed by using Shapiro-Wilk test, Levene's test, and Kruskal-Wallis test. Differences were accepted as statistically analysis at P <0.05.

3. Result

3.1. Phytochemical test

Phytochemical test results showed that Centella asiatica extract contained flavonoids, alkaloids, triterpenoids, glycosides, and tannins, while Coffea canephora extract contain alkaloids, tannins, saponins, flavanoids, and glycosides.

3.2. Average Measurement Results of Hydration Levels

In this study, examinations were carried out 5 times in all treatment groups: Initial before treatment, 1 week after treatment, 2 weeks after treatment, 3 weeks after treatment, and 4 weeks after treatment. The test of data normality was performed by using the Saphiro-Wilk test and the results showed that the research data was not normally distributed ($p < 0.05$). The measurements of average hydration levels in all treatment groups are summarized in table 2.

According on table 2, the best result of the hydration level concentration was found on 10% of Coffea canephora extract and 10% Centella asiatica extract treatment group at the last week the measurement time,, while the lowest result was present at the initial measurement time. The hydration level of each groups was increased by the addition of the treatment time.

Table 2: Measurement of The Average Hydration Levels in All Treatment Groups

Test Group	**Measurement time**	***Mean***	***95% CI***	***Max***	***Min***
Kontrol	Initial	27,1	25,6-28,6	30	24
	Week 1	27,2	25,8-28,6	30	24
	Week 2	28	26,6-29,4	31	25
	Week 3	28,3	27,1-29,5	31	26
	Week 4	29,1	27,6-30,6	32	26
Centella Asiatica 10%	Initial	27,0	25,4-28,5	30	24
	Week 1	29,9	27,8-31,9	35	27
	Week 2	33,4	30,9-35,9	40	29
	Week 3	37,3	34,8-39,9	44	33
	Week 4	42	39,5-44,5	48	38
Coffea canephora 10%	Initial	26,5	25,5-26,5	29	25
	Week 1	30,2	28,6-31,8	33	27
	Week 2	35,2	33,5-36,9	39	32
	Week 3	40	37,6-42,4	45	36
	Week 4	44,5	41,9-47,1	50	41

3.3. Hydration Percentage Levels

percentage increase in hydration levels of each groups of sample data can be seen in table 3 which performed by Kruska-Wallis test.

Table 3: Increased of Average Difference in Measurement of Hydration Levels on Each Groups

Target	**Test Groups**	***Mean* (N=27)**	***SD* (N=27)**	***Mean Rank***	**P**
Hydration level	Control	43,6	27,4	5,00	0,000*
	Centella Asiatica 10%			15,28	
	Coffea canephora 10%			21,72	

Based on table 3, the mean percentage increase in skin hydration levels in all treatment groups was 43.6% with a standard deviation of 27.4. The percentage difference in the increase of skin hydration level between groups was significant ($p < 0.005$). The best result of skin hydration percentage level was found in the 10% Robusta coffee treatment group followed by 10% gotu kola extract and control.

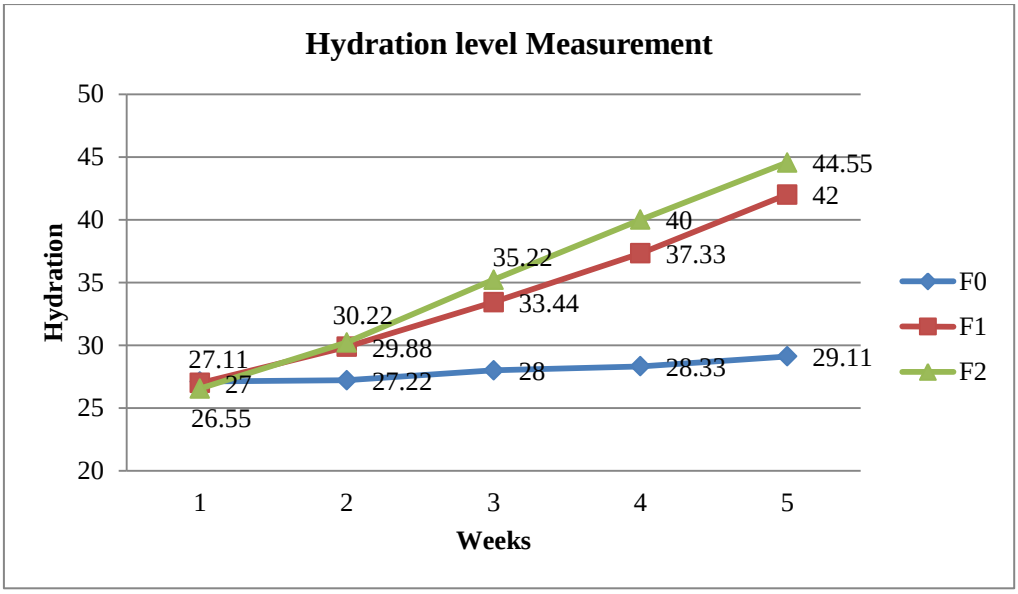

Figure 1: Graphics of Hydration Levels on Each Weeks

Based on figure 1, Showed that the application of cream with or without any extract still increase the hydration levels for four weeks of treatment time. However, cream with 10% of Robusta coffee extract showed the best result of the increase on hydration levels by 67.64% with an average final value of 44.55 (very high), whereas creams with gotu kola herbal extract 10% showed an increase in hydration levels as well as an average of 55.84 % with an average final score of 42 (very high), and control creams showed an increase in hydration levels by an average of 7.64% with an average final score of 29.11 (very less) during the four weeks of treatment.

4. Discussion

Phytochemical examination result show that Centella asiatica or gotu kola extract contain alkaloids, tannins, triterpenoids, flavanoids, and glycosides, while Coffea canephora extract contain alkaloids, tannins, saponins, flavanoids, and glycosides. The content of triterpenoids, flavonoids and glycosides in centella asiatica which is mostly glucose serves to bind water so that moisture in the skin will be maintained, besides that flavonoids as antioxidant compounds can increase extracellular collagen, which increases the level of moisture and elasticity in the skin, which increases the level of moisture, inhibits lipid peroxidation reactions and good reducing compounds, besides that flavonoids also act as a good inhibitor for hydroxide radicals and superoxide that protects the lipid membrane so that it can cause reduced pore size and improve skin texture [17]. This study has same result with research conducted by Milani and his colleagues [18], the daily application of fluids containing hyaluronic acid, glycerin, and centella asiatica extract induces a lasting hydration and moisturizing effect (up to 24 hours), at the same time also improves skin barrier function in healthy women. Glycerin and hyaluronic acid are two potential humectant agents. Hyaluronic acid can be degraded by the hyaluronidase enzyme. While the enzymatic activity of hyaluronidase can be inhibited by the extracts from centella asiatica, thereby increasing the duration and levels of skin hydration through inhibition of degradation of hyaluronic acid. Lyko and his colleagues [19] evaluated the effect of cosmetic formulations containing various concentrations of Centella asiatica extracts (2.5 and 5%) on skin hydration, Transepidermal Water Loss (TEWL) and micro-inflammation

in human skin using in vivo techniques (corneometer , tewameter and chromameter) for four weeks with two applications per day. In vivo test results containing 5% of gotu kola extract indicate the highest effectiveness in reducing TEWL, improve skin surface hydration which will also increase skin moisture, and showing anti-inflammatory effects of methyl nicotinate based models of micro-inflammation in healthy human skin. Yulianti and his colleagues [20] conducted a study evaluating skin cell proliferation, collagen synthesis and AQP3 expression in vitro against gotu kola ethanol extracts which were packaged in chitosan nanoparticles. In this study, the result showed that the extract could be used as active formulations for new anti-aging cosmetics that were new regulates skin hydration by increasing skin cell proliferation and AQP3 gene expression (Aquaporin 3). The results of this study also have the similar results with the study conducted by Fukagawa and his colleagues [21] who investigated the effects of coffee polyphenol (CPP) compounds on the skin and the function of microcirculation in humans. This research was conducted by giving a test drink containing CPP (270 mg / 100 mL / day) or a placebo drink for 8 weeks to 49 female subjects with mild xerotic skin. CPP consumption significantly decreases clinical rates for skin xerotics, decreases in skin surface pH, transepidermal water loss, and can increase stratum corneum hydration and responsiveness of skin blood flow during local heating. In addition, after consuming CPP, the amount of lactic acid and free fatty acids in the stratum corneum also increased significantly. The results of this study indicate that the function of skin permeability and hydration can be improved by intake of CPP for 8 weeks, together with improvements in the function of microcirculation, which leads to effectiveness in the improvement of mild xerotic skin. In another research, Rodrigues and his colleagues [22] conducted an in vivo study of the inhibitory effect of hyaluronidase. This test was carried out using a coffee skin extract based cream, twice a day, for more than 28 days in 20 human volunteers. After that, compare the coffee-containing cream with a formulation added to 1.5% of HyaCare® Filler CL (cross-linked polysaccharides made from hyaluronic acid derived from fermentation) by measuring the hydration and firmness of the skin. Coffee skin extract is proven to be an effective ingredient in improving skin hydration and firmness with results similar to hyaluronic acid. In the research of Kim and his colleagues [23], caffeic acid can improve keratinocyte differentiation and restore skin barrier homeostasis and it is recommended to be an appropriate skin therapy agent to improve the barrier function of epidermal permeability. Because of their high antioxidant activity, caffeic acid and chlorogenic acid protect organisms from free radicals and can be used in cosmetic products for skin applications to keep skin healthier and younger looking by avoiding reduced skin hydration, pigmentation, fine wrinkles, signs of aging and possible exposure to neoplasm [24].

5. Conclusion

The result of this study indicate that the highest percentage of skin hydration levels was found in 10% Robusta coffee treatment group followed by 10% Centella asiatica extract and control. The mean percentage increase in skin hydration levels in all treatment groups was 43.6%. The percentage difference in the increase in skin hydration levels between treatment groups was significant ($p < 0.005$).

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