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Effectiveness Test of Robusta Coffee (*Coffea cenephora)* Extract from North Sumatra in Collagen and Hydration Skin Level of Female Wistar *Rattus norvegicus*

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Abstract

Robusta coffee (Coffea canephora) is one of the Indonesian plants that have antioxidant compounds that can be used as cosmetic ingredients. The antiaging effect of the coffee extract can overcome the photoaging problem. The purpose of this study is to see the anti-aging effects of robusta coffee bean extract based on concentration variations of the female white rats. The study was conducted in a laboratory with pre and post control group design, from February to March 2019. 30 female white rats were divided into 5 groups and were sunbathed for 5 days, then were shaved 2x2 cm wide on the back and were given Robusta coffee bean extract cream 2.5%, 5%, 7.5%, 10%, and control. Applying cream twice a day for 4 weeks and changes were measured every 1 week for 4 weeks with a skin analyzer. The results were tested for the normality with the Shapiro-Wilk test and Levene's test. Then followed by Anova Repeated, Pearson Correlation and Multiple Linear Regression test. Data analysis was using SPSS 21.0. There were the highest increased of collagen and hydration levels with Coffea canephora 10% extract given, and variable concentrations and duration of administration have a positive effect. The highest increased of collagen level was in the Coffea canephora 10% extract with an average 91.3% and hydration level amounted to 86.09%.

Keywords: Coffea Cenephora; Collagen; Hydration; Anti-Aging.

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1. Introduction

Skin is the most outer part of the body and has a very important role such as protection, body temperature regulation (thermoregulation), excretion, absorption, perception, pigment formation (melanocytes) and keratinization. The skin consists of epidermis, dermis and hypodermis layers. Health and life conditions can be reflected from the skin condition [1]. The skin aging process consists of 2 categories such as intrinsic and extrinsic aging. Intrinsic aging is characterized by skin atrophy with loss of slow elasticity metabolic activity. Extrinsic aging is caused by environmental factor, the long-term effect of repeated exposure to ultraviolet light is the most significant and is referred as photoaging [2]. UV light energy, especially UVB with the free radicals induced, can damage collagen synthesis, which in turn decreases in collagen number. These changes make the skin surface become sagging and reduce its elasticity and cause wrinkle. In the stratum corneum layer this exposure can change the mechanical barrier characteristic and function, resulting in the increased of transepidermal water content and hydration loss in the stratum corneum [3,4]. Age increased and estrogen hormone decrease which plays the role in the process of converting fibroblasts into collagen, resulting in reduced collagen number [5]. Anti-aging cosmetic products that can be used topically can relieve symptoms and slowing the onset of photoaging symptoms caused by UV light [6]. Coffee is popular plant and divided into two species of plants namely *Coffea arabica* and *Coffea cenephora* or better known as arabica and robusta. Recent study reveals that consuming coffee can reduce the prevalence of several diseases such as diabetes, cardiovascular disease, cancer and Parkinson's disease. Coffee consumption can also increase plasma antioxidant capacity. Coffee extract can be used as cosmetic and pharmacological ingredient [7]. Because of its high antioxidant activity, caffeine acid in coffee can be used in cosmetics to protect from free radicals that makes the skin healthier and look younger by maintaining the skin hydration, pigmentation, fine wrinkles, and treating skin infection such as acne and rosacea [8]. Pholyphenol, alkaloid, tannin and saponin that are found in *Coffea cenephora* have high antioxidant activity [9]. The purpose of this study is to see the anti-aging effect of robusta coffee bean extract (*Coffea cenephora*) based on concentration variations on the increased of collagen and hydration skin level of female wistar *Rattus norvrgicus.*

2. Material and Methods

The study method used is non-experimental and experimental study, using pre-test and post-test control group design. Non-experimental study include robusta coffee bean extract, phytochemical examination and anti-aging cream production preparation using robusta coffee bean extract with concentrations of 2.5%, 5%, 7.5%, and 10%. Experimental study includes anti-aging activity test with the tool.

2.1. Tools and Materials

The tool used was the Skin Analyzer EH 900 U. The materials used are aquades, Ethanol pa (Merck, 96%), Bouchardate reagent, Mayer reagent, Dragendorff reagent, HCL2N Solution, H2SO4 Solution, HCl Solution, Stearic Acid, Sodium Benzoate, Glycerin , Triethanolamine, Robusta coffee beans from Pematang Purba.

2.2. Extract Production

Coffee bean extract was made using robusta coffee bean (*Coffea cenephora*) which has been crushed to be fine powder. The extract was made by maceration technique with ethanol solution 96%. 120 gr coffee powder was soaked with 225 ml of ethanol 96% for 5 days in closed condition and was stirring occasionally. On the 6^{th} day the mixture extract and solvent were filtered with filter paper and produced filtrate 1 and grounds 1. Grounds 1 was soaked again with 75 ml of ethanol solution 96% for 2 days in closed condition and was stirring occasionally. Filtering was done after 2 days to separate filtrate 2 and grounds 2. Extract was obtained by combining filtrate 1 and 2 and then was evaporated with rotary evaporatory. The extract was stored and left at room temperature until the ethanol solvent evaporated and ready to be used for the test [10].

2.3. Phytochemical Test

Alkaloid examination was done with 100 mg of coffee beans extract in 3 tubes. Then 1 ml of HCl_2N and 9 ml of water were added and heated at 95^0C for 5 minutes, then were cooled and filtered. Each tubes were given 2 drops of Bouchardat, Mayer, Dragendorff reagent. Alkaloid test is positive if there is sedimentation or at least two or three of the trials [11]. The tanin examination was done with 100 mg of coffee bean extract, 100 ml of hot water was boiled for 5 minutes. The filtration product was filtered with two drops of $FeCl_3$ 1%. The tannin test is positive condensated with brownish green colour formed and the tannin test is positive hydrolyzed with blackish blue color formed [11]. Saponin examination was done with 100 mg of coffee bean extract added 10 ml of hot water, then was cooled, shaken strongly for 10 seconds. The saponin test is positive if foam formed which doesn't disappear before 10 minutes and after add 1 drop of HCl_2N the foam is also not go away [11]. Triterpenoid examination was done with 0.5 g of coffee bean extract was dissolved in 5 ml of chloroform, 5 ml of anhydrous acetate, and 2 ml of concentrated H_2SO_4 solution. Positive terpenoid test is showed by red, orange or purple colours formed [11]. Flavonoid examination was done with 100 mg of coffee bean extract added with 10 ml of hot water, was boiled for 5 minutes and was filtered. 5 ml of filtrate was pipetted and then added with 100 mg of magnesium powder, 1 ml of concentrated HCl and 2 ml of amyl alcohol and then shaken strongly. Positive flavonoid test has orange or reddish orange colours in the amyl alcohol layer. Flavonoid identification was done in a acid box [11]. The glycoside examination was done with ± 3 g coffee bean extract filtered with 30 ml of technical ethanol mixture with water (7: 3) then was refluxed for 10 minutes, was cooled and filtered. 20 ml of filtrate was added with 25 ml of distilled water and 25 ml of lead (II) acetate 0.4 M, was shaked, allowed to stand for 5 minutes then was filtered. The filtrate was extracted with 20 ml of chloroform and isopropanolol mixture (3: 2), was repeated for 3 times. The preparation was evaporated at temperature less than 50^0C and was dissolved in 2 ml of methanol and then was evaporated. Added 2 ml of water with 5 drops of molish reagent. Then slowly added 2 ml of concentrated sulfuric acid through the tubes wall. The glycoside test results is positive if there is purple ring formed in the second liquid boundary, indicating there is sugar bond [11].

2.4. Cream Production

Cream compositions used standard formula that used basic type of oil cream in water [12]. Cream was divided into 5 concentrations, cream compositons are attached in table 1.

Table 1: Cream Formulation

Composition	Consentration				
	F0	**F1**	**F2**	**F3**	**F4**
Coffea cenephora Extract	-	2,5%	5%	7,5%	10%
Stearat Acid	7,5g	7,5g	7,5g	7,5g	7,5g
Trietanolamine	0,75g	0,75g	0,75g	0,75g	0,75g
Natrium Benzoate	0,1g	0,1g	0,1g	0,1g	0,1g
Gliserine	5g	5g	5g	5g	5g
Aquadest	Ad 50	Ad 50	Ad 50	Ad 50	Ad 50

2.5. Animal Trial Procedure

Anti-aging activity study used 25 samples of white female wistar rats (*Rattus norvegicus*) and divided into 5 groups. Group 1 used F0 (control), group 2 used F1 (2.5% *Coffea cenephora* extract), group 3 uses F2 (5% *Coffea cenephora* extract), group 4 used F3 (7.5% *Coffea cenephora* extract) and group 5 used F4 (10% *Coffea cenephora* extract). The whole group of white rats were sunbathed previously for 5 days for 4 hours (9 am until 1 pm), then the hair on the back was shaved 2x2 cm^2 using an electric shaver and a manual hair shaver. Then the condition of the test animals were measured before treatment with Skin Analyzer EH 900 U including water and collagen level.After measuring, the cream was applied thinly and evenly on the shaved area for 2 times a day (8 am and 6 pm) for 4 weeks. Measurement of water and collagen level were done every weekend for 4 weeks with skin analyzer.

2.6. Statistical Analysis

Anti-aging activity of *Coffea cenephora* extract datas were analyzed with SPSS 21 program. Normality data test with Shapiro-Wilk test and homogenity data with Levene's test ($p > 0.05$). Data then were tested with Repeated Anova Test, followed by the Pearson Correlation and Multiple Linear Regression test. The differences were accepted as statistical analysis at $p < 0.05$.

3. Result and Discussion

3.1. Phytochemical Test Results

From the *Coffea cenephora* phytochemical test results were obtained extract contained alkoaloid, tannins, saponins, triterpenes/steroids, flavonoids and glycosides compounds. The results are attached in table 2.

Table 2: Phytochemical Test Results

Phytochemical Test	*Coffea cenephora*	Caption
Alkaloid	+	Sedimentation (+), two or three from the trials
Tannin	+	Violet green or blackish blue colours sedimentation (+)
Saponin	+	Permanent foam
Triterpen/Steroid	+	Red, orange, purple ring colours
Flavonoid	+	Orange or reddish orange fluoresense
Glycoside	+	Purple on both liquid boundary

3.2. Collagen Level Examination Results

The examination in this study was done for 5 times in each treatment groups. Examination before treatment, 1, 2, 3 and 4 weeks after treatment. Normality Test of the data was done with Shaphiro-Wilk test, was obtained normal distribution variables ($p < 0.05$). The results of collagen levels examination are attached in table 3.

Tabel 3: Collagen Level Test Results

Group	Treatment Duration					
	Early Condition	1st Week	2nd Week	3rd Week	4th Week	Increased Precentage
Control	24,4	24,8	25,2	25,4	25,4	4,09
F1	25,2	27,6	29,4	31,2	33,2	31,7
F2	26,2	28	29,6	35	41	56,4
F3	25,8	29,2	35,2	40,8	45,2	75,1
F4	27,8	30,8	38,2	43,8	53,2	91,3

The results showed that applying cream with or without *Coffea cenephora* extract for 4 weeks, both increased collagen skin level of female white rats. However, cream with *Coffea cenephora* 10% extract showed the highest increased in collagen level by an average of 91.3% with final average score 53.2 (good). Increasing collagen level significantly started from 1st week to 4th week (sig. 0.00). Both concentration and time variables have significant effect on the collagen level variables (sig 0.00). Another study was done by Safrida and his colleagues (2017) in aging premenopous rats were given coffee and ethinylestradiol extract resulting in higher collagen level and skin RNA level compared with control. In the measurement of water level, rats skin that were given coffee and ethinylestradiol had the same results with control. The trigonellin content in coffee extract can increase cell synthesis activity in rats. The conclusion of this study is that applying aceh arabica coffee extract on the aging skin increased collagen and skin RNA level [13]. Study was done by Handayati and his colleagues (2017), there was an increasing in collagen density level for 246.96% from using coffee paste 40% applied topically, compared with Vitamin C [14]. Another study was done by Goresselli and his colleagues (2017)

found that skin healing used Roasted Coffee Oil (RCO) was faster than Green Coffee Oil (GCO). High level of IGF-1 mRNA expression in animal skin after RCO administration on the fourth day were compared with the control. Both types of these coffee oil also have a systemic effect, although with topical administration. During the wound healing process, fibroblasts must produce and deposit collagen into the extracellular matrix. IGF-1 is a growth factor produced by fibroblasts and other epithelial cells, this has an important role in the process of re-epitalization and granulation tissue during the wound healing process. One of the role of vascular endothelial insulin/IGF-1 is by providing vascular homeostasis to the skin and neovascularization during the wound healing process. Because the role of IGF-1 in angiogenesis, increasing of IGF-1 level in early stage is a very important process [15]. Coffee contains more antioxidants than fruits and vegetables and can be used as antiphotoaging. Coffee antioxidant compounds include polyphenols, flavonoids, proanthocyanidin, coumarin, chlorogenic acid, trigonellin and tocopherols. This antioxidants stimulate collagen in the dermis layer by producing *Tissue Inhibitor Metallo-proteinas-1* (TIMP-1) on the skin which to inhibit collagen damage and plays role in the healing stage in every wound healing [14]. TIMP-1 is a glycoprotein, and work by inhibit the *matrix metalloproteinases* (MMPs) action. This compound degrades the skin protein matrix, such as collagen through enzymatic activity so it will interfere the dermis integrity which results in the skin damage [16].

3.3. Hydration Level Examination Results

In this study the examination was done for 5 times in each treatment group. Normality Test was done with Saphiro-Wilk test resulted in normally distributed variables ($p < 0.05$). The results of the hydration level are attached in table 4.

Tabel 4: Hydration Level Test Results

Group	Treatment Duration					
	Early Condition	1st Week	2nd Week	3rd Week	4th Week	Increased Precentage
Control	28,4	28,8	29,2	29,8	30,6	7,74
F1	28,2	28,8	32,0	34,2	35	24,11
F2	28,8	30,0	35,4	40,4	44,4	54,16
F3	30,6	31,4	38,2	44,6	52,0	69,9
F4	30,2	31,2	38,6	46,2	56,2	86,09

The results showed that applying cream with or without *Coffea cenephora* extract increased hydration level during four weeks of treatment. However, the cream with *Coffea cenephora* 10% extract showed the highest increased in hydration level with an average of 86.09% with final value average of 56.2 (very high). Another study was done by Putri and his colleagues (2019) found that skin hydration level with applying robusta coffee 10% extract cream was higher than *Centella asiatica* 10% extract cream. The study was done for 4 weeks and was found an increased average percentage of 43.6% in skin hydration. The percentage increased in skin hydration level in each group had a significant result ($p < 0.005$) [17]. Research was done by Ribeiro and his colleagues (2012) using cream containing 10% of lipid fraction from Spent Coffee Grounds (SCG), was

extracted with carbon dioxide which showed lipid quality (sebum) and improved hydration quality [18]. The results of this study are also in accordance with Fukugawa and his colleagues (2017) to see the effect of drinking polyphenol coffee (CPP) in skin tissue and microcirculation function in human. From the study result it was found that the group consuming CPP significantly reduced xerosis skin condition, decreased Transepidermal Water Loss (TEWL), skin surface pH level, increased hydration of the stratum corneum and responsed to the blood flow in skin [19]. High antioxidant content in coffee, can block free radicals and good cosmetic ingredients to maintain healthy skin, help rejuvenation by avoiding decreased of skin hydration, pigmentation and dark spots. As an anti-bacterial caffeic acid can be used to treat skin infection such as acne and rosacea [20]. As a cosmetic ingredient, caffeine is used as an active anti-cellulite ingredient because it can prevent the accumulation of excess fat in the cells. These alkaloid can stimulates fat degradation during lipolysis by inhibiting phosphoesterase activity. Caffeine has strong antioxidant characteristic. Caffeine in coffee can protect the skin against UV radiation and inhibit the aging skin process [21].

4. Conclusion

From the study results were obtained *Coffea cenephora* 10% extract cream showed the highest increased of collagen level (91.3%) and hydration level (86.09%). While *Coffea cenephora* 2.5% extract cream showed the lowest increased in collagen level (31.7%) and hydration level (24.11%). Increased of collagen and hydration levels were seen starting from 1st week to 4th week, as well as the concentration and duration (time) variables giving a positive effect on increasing collagen and hydration levels (sig < 0.05).

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References

[1] Wasitaatmadja SM. 2013.Faal Kulit. In : Adhi D,editor. Ilmu Penyakit Kulit dan Kelamin.Jakarta : Fakultas Kedokteran Universitas Indonesia .p.7-8.

[2] Sjerobabski-Masnec I, Situm M. 2010. Skin aging. Acta Clin Croat. Dec;49(4): 515-8. PubMed PMID:21830465.

[3] Biniek,K.,Kemal.L & Reinhold.H.D. 2012. Solar UV radiationreduces the barier function of human skin. In : John W.H (ed) Proceedings of the National Academy of Sciences of the United States of America. 109 (42), National Academy of Sciences, pp 17111–17116. doi: 10.1073/pnas.1206851109.

[4] McLafferty E, Alistair F, and Hendry C. 2012. The integumentary system: anatomy, physiology and function of skin . Nursing Standart ; Vol. 27 (3): p.35-42.

[5] Alhana, Pipih S, Kustiariyah. 2015. Extraction and Characterization of Collagen from Sea Cucumber Flesh.JPHPI. Aug; Vol 18(2): 150-161.

[6] Barel A.O., Paye M. and Maibach H.I. 2009. Handbook of Cosmetic Science and Technology. 3rd Edition. New York : Informa Healthcare USA Inc.

[7] Chaiyasut C, Bhagavathi SS, Sisithorn S, Netnapa M, Jakkapan S, Sartjin P,et al. 2018. Formulation and Stability Assessment of Arabica and Civet Coffee Extraccts Based Cosmetic Preparation. Asian Journal of Pharmaceutical and Clinical Research. June ;Vol 11:425-429.

[8] Magnani C, Thais SO, Vera LI, Marcos AC, Herida RS. 2015. Validation of Caffeic Acid in Emulsion by UV-Spectrophotometric Method. Journal Physical Chemistry .5(1):16-22.

[9] Chairgulprasert.V and Kittiya.K. 2017. Preliminary Phytochemical Screening and Antioxidant Activity of Robusta Coffee Blossom. Thammasat International Journal of Science and Technology. 22(1):1-8.

[10] Tanuma HA, Gayatri C, Widya AL. 2016.Aktivitas antibakteri ekstrak biji kopi robusta (Coffea cenephora) terhadap bakteri Escherichia coli.Jurnal Ilmu Farmasi. 5(4):243-251.

[11] Ciptaningsih,E. 2012. In Vitro Antioxidant Activity and Phtochemical Characteristics of Kopi Luwak and Its Effect on Blood Pressure in Normotensive and Hyoertensive Rats. Master of Pharmaceutical Sciences Thesis.Jakarta: University of Indonesia.

[12] Sharma GK, Gadiya J, and Dhanawat M. 2016.Textbook of Cosmetic Formulation. India: Kbuuk Publication.

[13] Safrida and Mustafa Sabri. 2017.Potential Of Aceh Arabica Coffee Extract (Coffea arabica L) in Rejuvenation of Aging Skin Rat.Jurnal Kedokteran Hewan.11(3):101-103.

[14] Handayati D, Fifa A, Yuli K, Citra TM, Yudha DS. 2017.Efficacy of Topical Coffee Paste to Colagen Density in Wistar Rats Incision Wound. Bioscientia Medicina.2(1):12-15.

[15] Grosselli B, Joseane M, Anglecio LS, Marilene NS, Amanda RA, Gislaine V, et al. 2017.Topical use and systemic action of green and roasted coffee oils and ground oils in a cutaneous incision model in rats (Rattus norvegicus albinus).In. Tatiana.M (Ed).PLOS.1-17.

[16] Alam M and Havey J. 2010. Chapter 2: Photoaging . Cosmetic Dermatology: Products and Procedures. New York: Blackwell Publishing.

[17] Putri E, Celvin A, Chrismis NG, Linda C, I Nyoman EL. 2019. 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. American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS).61(1):192-201.

[18] Ribeiro H, Joana M, Sara R, Maria A, Vera I, Bruna GC, et al. 2012. From coffee industry waste materials to skin-friendly products with improved skin fat levels. Eur. J. Lipid Sci. Technol.115:330–336.

[19] Fukagawa S, Satoshi H, Shun S,Yuka Y, Hisashi T, Takatoshi M. 2017. Coffe Polyphenols Extracted From Green Coffee Beans Improved Skin Properties and Microcirculatory Function. Biological Science Laboratories, Kao Corporation, Tochigi, Japan. Bioscience, Biotechnology, and Biochemistry Journal.1814-1822.

[20] Magnani C, Thais SO, Vera LI, MarcosAC, Herida RS. 2015.Validation of Caffeic Acid in Emulsion by UV-Spectrophotometric Method. Journal Physical Chemistry .5(1):16-22.

[21] Herman A, AP Herman. 2013.Caffeine's Mechanisms of Action and its Cosmetic Use.Journal Skin Pharmacology and Physiology.Vol 26:8-14.