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STUDI BIOAKTIF ANTI AGING PADA BIJI KOPI ARABIKA (*COFFEA ARABICA*), ROBUSTA (*COFFEA CANEPHORA*), DAN LIBERIKA (*COFFEA LIBERICA*) JAMBI

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CHEMICAL STUDY OF THE ETHANOL EXTRACT OF ARABICA COFFEE (COFFEA ARABICA), ROBUSTA (COFFEA CANEPHORA), AND LIBERICA (COFFEA LIBERICA) JAMBI THAT HAS THE POTENTIAL ANTIAGING ACTIVITY

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1. Introduction

The aging process is a physiological process that cannot be avoided and affects all organs of the body including the skin. Sun exposure generally consists of ultraviolet (UV) rays which contribute to making the skin wrinkled, rough and also experiencing thickening (Rabe, 2005). DNA is the main molecule that regulates the function of every cell in the body. According to dermatologists, the main target that is damaged due to UV light exposure is cell DNA (Leynor, 2007). The impact of DNA damage by UV rays can be seen from the damage to the growth of cells.

Skin that is exposed to repeated UV rays produces MMP expression in RNA and protein which causes premature aging. Mice that are given regular UV exposure cause thickening of the epidermis of the skin, resulting in skin wrinkling and gelatinase activity from MMP-2 and MMP (Park, 2005).

Exposure to UV rays, namely UV B or UV A wavelengths on the skin, triggers the synthesis and transfer of melanin into keratonocytes. This results in thickening of the epidermis of the skin. Melanocytes are dendrite cells that are near the basal layer of the stratum germinativum. Melanocytes will synthesize melanin, a large polymer that is bound to proteins. This melanin absorbs light with very large wavelengths between 200-2,400 nm, so it is a good screen to protect the destructive effects of UV rays. The keratinization process also acts as a physiological mechanical protection and lasts a lifetime (Dharmojono, 2005).

Antioxidants have been shown to help reduce the skin's intrinsic (natural) signs of aging while restoring and protecting the skin from extrinsic (environmental) aging. Natural antioxidants (applied topically or ingested) can strengthen the activity of the body's endogenous antioxidant defense systems, thus providing additional protection from oxidative stress. McDaniel (2009) found that chlorogenic acid compounds, condensed proanthocyanidin, quinic acid, and ferulic acid in arabica coffee resulted in an increase in the accumulation of structural

proteins such as collagen I and collagen IV, as well as a reduction in collagenase (MM P-1) and IL-1b1 (inflammatory mediators).

The use of natural ingredients from plants for the treatment of diseases is still widely practiced, this is based on the consideration that the side effects of natural medicines are smaller than pure chemical drugs. In addition, coffee is one of the agroindustry commodities in Jambi Province. However, the fundamental problem in the coffee bean roasting process is the accuracy in selecting the roasting time (t) and temperature (T) in the thermal process that matches the characteristics of the coffee beans. Roasting temperature and time greatly affect chemical and physical changes which generally result in changes in chemical composition, namely: (i) changes or degradation of polysaccharide compounds, (ii) decreases or increases in caffeine content, (iii) decreases in protein content, (iv) decreases in compounds -compounds that are not palafified and (v) increase in free fatty acids. The main constituent elements of coffee beans are Carbon, Hydrogen, Nitrogen, Oxygen and various other minerals and metals.

According to Mulato (2002) states that coffee beans naturally contain various types of volatile compounds such as aldehydes, furfural, ketones, alcohols, esters, formic acid, and acetic acid which have volatile properties. Compounds that cause a sharp or sour taste such as tannins and acetic acid will be lost and some will react with amino acids to form melancidin compounds which give a brown color. The results of preliminary analysis studies using LC-MS on Liberica, Arabica, and Robusta coffees show that the roasting process causes changes in the composition of the compounds contained in coffee. In Liberika coffee after roasting, there are no derivative compounds of chlorogenic acid, quinic acid, and ferulic acid (Perdana et al, 2018; Heriyanti et al, 2019). Green arabica coffee contains chlorogenic acid and green robusta coffee contains ferulic acid derivatives. Meanwhile, roasted robusta coffee contains derivative compounds of ferulic acid and quinic acid (Sutrisno et al, 2020).

The content of chlorogenic acid, quinic acid, and ferulic acid derivatives contained in coffee has the potential to be used for anti-aging because of its antioxidant properties that can bind to free radicals. These antioxidant properties can also strengthen the activity of the body's endogenous antioxidant defense system, thus providing additional protection from oxidative stress, thereby preventing premature aging.

McDaniel (2009) explains that phenolic compounds extracted from Arabica coffee can reduce levels of MMP-1 and IL-1b. In addition, upregulated gene expression for four collagen structural proteins and derived gene expression for three MMPs were also observed to infer the reparative effect of Coffea arabica extract on photoaged skin.

Preliminary studies that have been conducted have shown that arabica, robusta, and liberal coffee contain derivatives of chlorogenic acid, ferulic acid and caffeine which are antioxidants and can be used as anti-aging (Perdana et al, 2018; Heriyanti et al, 2019).

Information about the compound content of robusta and arabica coffee beans as well as liberica on methanol and fraksinate extracts has not been studied in more depth. Based on these reasons, it is necessary to isolate and characterize antiaging active compounds. The results are expected to be able to answer the types or groups of active compounds that are anti-aging and at the same time know the characteristics of the active compounds as anti-aging candidates.

2. Materials and Methods

Materials

The coffee beans used in this research were robusta, liberica and arabica coffee collected from Kerinci Regency (Robusta and Arabica) and Liberika from Tanjung Jabung Barat Regency, Jambi Province. The solvents used for extraction and chromatography were technical solvents that have been distilled, namely; methanol, n-hexane, benzene, diethyl ether, methylene chloride, acetone, ethyl acetate and chloroform. Vacuum liquid chromatography with Merck 60 GF254 silica gel, gravity chromatography with Merck 60 silica gel (230 - 400 mesh), and purity analysis of compounds by thin layer chromatography on plates coated with Merck 60 GF254 silica gel, 0.25 mm will be carried out according to the procedure standard.

Determination of the melting point was carried out with the Fisher John melting point apparatus, for the prospective chemical structure determination of compounds as antibiotics, UV-Vis, IR, NMR and MS spectroscopy was required. To test the antibiotic activity, tools such as petri dish, 37^{o}C incubator, autoclave, laminar air flow cabinet, loop, ELISA plate and other equipment commonly used in microbiology labs were used. The incubation site used in the antibiotic activity test was CO_2 incubator. Examination of the effect of active compounds on mice and their effects on the host tissue was carried out under the observation of Electron Microscopy.

Methods

- **Extraction and Isolation**

A total of 50 g of roasted Arabica, Robusta, and Liberika coffee were extracted with methanol as a solvent, followed by fractionation using ethanol, n-hexane, dichloromethane and ethyl acetate as solvents. The initial methanol extract was also tested for antibiotics against ethanol fraksinate, n-hexane, dichloromethane and ethyl acetate. For active extracts and

fraksinates, it was continued for determination or phytochemical testing. For fraksinates that contain chlorogenic acid, condensed proanthocyanidin, quinic acid, and ferulic acid and are active as antibiotics followed by isolation of chlorogenic acid compounds, condensed proanthocyanidin, quinic acid, and ferulic acid by performing TLC, gravity column chromatography and KVC. Each stage of isolation was guided by activity tests on mice. The isolates were determined by the type and nature of the compounds and their characteristics using spectroscopy, namely UV-Vis, FTIR, NMR and MS spectroscopy.

- **Antioxidant activity test**

DPPH method (Selvi et al., 2003). 10 mg of the sample was dissolved in 10 ml of methanol in a 10 ml volumetric flask, so that a concentration of 1 mg / ml was obtained for the determination of antioxidant activity, 0.2 ml of the sample solution was piped with a micro pipette into the vial, then added 3.8 ml of 50 µM DPPH solution. The solution mixture was homogenized and left for 30 minutes in a dark place. Absorption was measured by a UV-Vis spectrophotometer at a wavelength of 517 nm. For positive control, α-tocopherol was used the same treatment as the sample.

NBT / XOD method (Hodgson and Fridovich, 1976 in Degaulejac et al, 1999). Tetrazolium blue solution (NBT, 10-3 M) was prepared in a sodium phosphate buffer T (0.05 M; pH 7.4). Hypokxantin solution (HPX, 0.5 10-2 M) and xanthine oxidase (XOD, 1.67 units mL-1) were dissolved with T buffer. The test sample was dissolved in H_2O.

Inhibition test of xanthine oxidase activity (Hatano et al., 1990 in McCune et al., 2002). 12.5 µl of various concentrations of samples were added to the xanthine osidase solution 0.049 units/ml. The mixture was incubated for 20 minutes at 37^{o}C. After incubation, 250 µL of 0.40 mmol/L substrate was added to the mixture and incubated again for 20 minutes at 37°C, after which incubation was stopped by adding 500 µL SDS. The blanks were prepared in the same way, only the enzyme solution was added after adding the SDS. The test solution was measured at a wavelength of 290 nm using a spectrophotometer.

Preparation of Anti-aging of the ethanol extract of Arabica coffee, Liberika, and Robusta. Preparation of cream preparations was carried out by melting the oil phase, then adding to the water phase with an emulsifier, and stirring until a mass of cream mixture forms (Syamsuni, 2006). The oil phase consists of stearic acid, cetyl alcohol, and propyl paraben. The water phase consists of triethanolamine, glycerin, methyl paraben and aquadest. Each phase was melted at 70 ° C on a hot plate. After melting, the oil phase is slowly added to the water phase at 70 ° C while continuing to stir. The two masses are crushed in a heated mortar to form

a creamy mass. After it is cool enough, then add the extract of arabica coffee, liberica coffee, and robusta coffee slowly into the cream base while continuing to stir until it is homogeneous. The formula for the antiaging cream is presented in table 2.

Table 2. The formula for the antiaging cream

Material	Weight (%)		
	F1	**F2**	**F3**
Arabica Coffee, Liberika Coffee, Robusta Coffee	1	3	5
Stearic acid	10	9	8
Cetyl alcohol	3	4	5
TEA	1,5	1,5	1,5
Glycerine	1,8	1,8	1,8
Methyl Paraben	0,3	0,3	0,3
Propyl Paraben	0,3	0,3	0,3
Aquadest	Ad 100	Ad 100	Ad 100

3. Result and Discussion

The sample extraction process (Arabica, Robusta, and Liberica) was carried out by maceration using methanol, then partitioned with n-hexane and ethyl acetate. Separation of chemical compounds for all fractions was carried out using isolation techniques. The initial stage is to separate using KVC (Liquid Vacuum Column) and gravity chromatography (KKG) column. Silica gel for column packing was activated at 1100C for 15 minutes. Every 15 grams of the sample is impregnated with 15 grams of silica gel. Column packing is done by wet.

The ethyl acetate fraction of Robusta coffee was separated with KVC (Liquid Vacuum Chromatography) (Si gel 200 g) and eluted with a mixture of n-hexane, n-hexane - ethyl acetate, ethyl acetate-methanol whose polarity was increased gradually (n-hexane 100% to methanol 100%) and obtained 22 vials. TLC test results are presented in Figure 1.

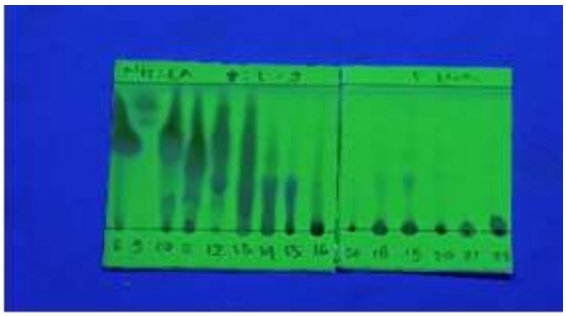
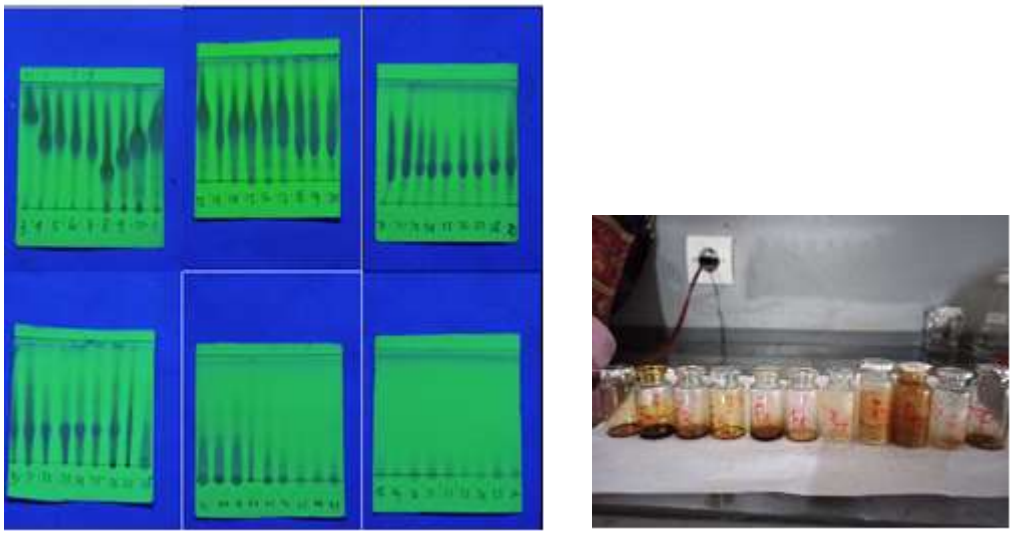


Figure 1. TLC chromatogram from KVC Ethyl Acetate Fraction of Robusta Coffee

Based on the chromatogram at KLT, the vials that have the same stain pattern were combined into one fraction group. The combined results obtained were F1 (vials 1 - 13), F2 (vials 14 - 15), and F3 (vials 16-22). Fraction 1 Ethyl Acetate (F1), continued the separation

using KKG. A total of 2 grams of F1 Ethyl Acetate extract, impregnated. Furthermore, elution was carried out using a solvent based on differences in polarity. The elution used for elution starts from hexane: ethyl acetate, 100% ethyl acetate, ethyl acetate: methanol, and 100% methanol. The elution results were obtained as many as 73 vials. The results of the KKG are monitored by TLC, as shown in Figure 2.



a) b)

Figure 2. (a). TLC chromatogram from the ethyl acetate F1 KKG (b). Vial of ethyl acetate F1 KKG results

Referring to the stain pattern shown from the TLC results, the 73 vials collected from the KKG were grouped into 7 subfractions, namely F1.1 (vial 3), F1.2 (vial 4-7), F1.3 (vial 8), F1. .4 (vials 9-2), F1.5 (vials 23-28), F1.6 (vials 29-36), and F1.7 (vials 37-73).

Subfraction F1.5 indicates the presence of sediment. The F 1.5 precipitate was separated with the solution. Furthermore, purification is carried out using the recrystallization technique. Furthermore, isolate F1.5. This purity was tested by TLC using 3 different eluents, to prove its purity, as shown in Figure 3.

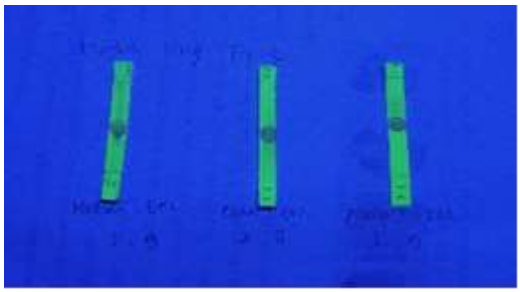


Figure 3. Results of klt F1.5 with hexane: ethyl (1: 9), dcm: ethyl (2: 8), and acetone: ethyl (1: 9) eluent

The TLC results in Figure 3 show 1 spot, for all the eluents used, this indicates that the isolate F 1.5 is pure. The next stage is the phytochemical test of isolate F 1.5, to determine the

class of its compounds. Furthermore, UV, IR and NMR spectroscopy were measured so that isolates could be characterized, based on the spectra of the spectroscopic measurements.

The isolate obtained was F 1.5, then tested for its phytochemical content using phytochemical reagents. The test result data is shown in Table 1.

Table 1. Phytochemical test results of Isolate F 1.5

Secondary metabolites	Reagent	Result
Alkaloid	Mayer	+
	Dragendorf	+
Phenolic	FeCl3 1%	-
Flavonoids	Mg+HCl+ ethanol	-
Terpenoids	Lieberman Burchard	-
Steroids	Lieberman Burchard	-
Saponins	Warm water	-
quinone	NaOH 1N	-

Results of Spectroscopic analysis

The identification of isolate F 1.5 was then analyzed based on testing using UV-Vis (Ultra Violet-visoible) and Fourier Transform Infrared (FTIR) spectrophotometric instruments. The spectra of the results of the UV-vis and IR analysis of Isolate F.4 are shown in Figures 4 and 5.

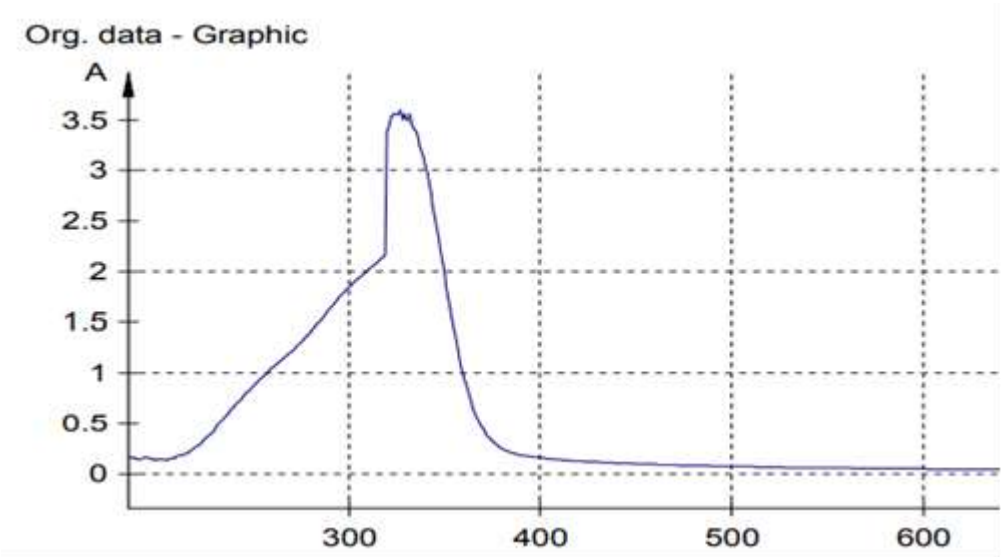


Figure 4. UV-Vis spectrum of isolate F 1.5

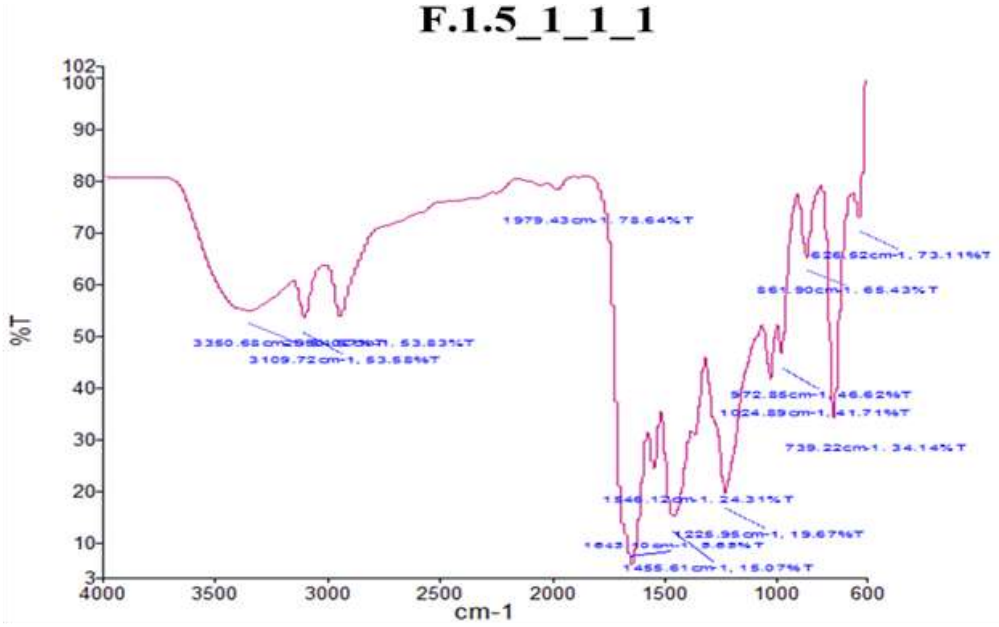


Figure 5. IR spectrum of isolate F 1.5

Test results of coffee extract cream

The test consisted of organoleptic test, pH, spread ability, homogeneity, and cream type. The test results are presented as follows. The parameters observed in this organoleptic were aroma, color, texture and consistency of the preparation. The results of observations on physical appearance (organoleptic) showed that the dosage forms of all formulas had cream forms (semi-solid) according to the specifications made. Organoleptic observations were carried out visually using the five senses to describe the sunscreen cream preparations that had been made. The organoleptic test result is presented in table 3.

Table 3. Organoleptic Evaluation

	R1	R2
F1	**Aroma** : Coffee **Color** : Pale brown **Texture** : Semi solid **Consistence** : Soft	**Aroma** : Coffee **Color** : Pale brown **Texture** : Semi solid **Consistence** : Soft
F2	**Aroma** : Coffee **Color** : Light brown **Texture** : Semi solid **Consistence** : Soft	**Aroma** : Coffee **Color** : Light brown **Texture** : Semi solid **Consistence** : Soft
F3	**Aroma** : Coffee **Color** : Dark brown **Texture** : Semi solid **Consistence** : Soft	**Aroma** : Coffee **Color** : Dark brown **Texture** : Semi solid **Consistence** : Soft

Based on the table above, it can be seen that Robusta coffee in all formulas has a distinctive aroma of Arabica coffee, semi-solid texture and soft consistency. While the F1 color has a pale brown color, while the F2 has a light brown color and F3 has a dark brown color.

The evaluation of the pH value is carried out to determine the pH value of the cream preparation which will affect the safety and comfort of using the preparation on the skin. Following are the results of the evaluation of the pH value of the cream preparations are presented in the Table 4.

Tabel 4. Coffee pH Test Results

Arabica Coffee			
	F1	F2	F3
R1	7,0	6,6	5,9
R2	6,9	6,5	6,0
Liberica Coffee			
	F1	F2	F3
R1	6,6	6,5	6,3
R2	6,8	6,4	6,1
Robusta Coffee			
	F1	F2	F3
R1	6,7	6,3	6,3
R2	6,8	6,2	6,3

Based on the table 4, the pH results obtained by each coffee preparation from 3 different variations have a pH range of 5.9-7.0. This shows that the three coffee preparations have a pH that is safe and according to the standards based on the requirements of SNI 16-4954-1998, which ranges from 3.5-8. So it is hoped that the formulated cream will not irritate the skin.

The spread ability evaluation was conducted to determine the spreading ability which affects the comfort of the cream when applied to the skin. In this study, the observed parameter to measure the spread ability of the cream preparations was to measure the diameter of the spread of the cream resulting from the application of a certain load. The data from the evaluation of the spread ability are presented in the table

Tabel 5. Result of Spread ability Test

Arabica Coffee			
	F1	F2	F3
R1	5,9 cm	5,7 cm	5 cm
R2	6 cm	5,5 cm	5 cm
Liberica Coffee			
	F1	F2	F3
R1	6,8 cm	6,5 cm	5,3 cm
R2	6,7 cm	6,3 cm	5,5 cm
Robusta Coffee			
	F1	F2	F3
R1	7 cm	6,5 cm	6 cm
R2	7 cm	6,4 cm	6,1 cm

Based on the table above, the dispersion power value obtained is in the range of 5-7 cm. This value indicates that the spread ability of all formulas has a good value because

according to Garg et al (2002), the value of the spread ability ranges from 5-7 cm. Therefore, a good cream preparation must meet the predetermined dispersion evaluation requirements. The spread ability test is used to describe the ease with which the cream is applied to the skin.

Homogeneity evaluation aims to determine the distribution of active substances in cream preparations where the cream must have a homogeneous structure and do not show any spots and coarse particles. The results of the homogeneity evaluation of the cream are presented in the Table 6.

Table 6. Result of Homogeneity Test

Arabica Coffee			
	F1	F2	F3
R1	homogeneous	homogeneous	inhomogeneous
R2	homogeneous	homogeneous	inhomogeneous
Liberica Coffee			
	F1	F2	F3
R1	homogeneous	homogeneous	inhomogeneous
R2	homogeneous	homogeneous	inhomogeneous
Robusta Coffee			
	F1	F2	F3
R1	homogeneous	homogeneous	inhomogeneous
R2	homogeneous	homogeneous	inhomogeneous

Based on the table 6, for each Arabica coffee, Liberica coffee and Robusta coffee in formula 3 the cream preparation is not homogeneous. Meanwhile, formula 1 and formula have homogeneous preparations. According to the Indonesian Ministry of Health (1985), the cream must show a homogeneous structure and do not show any spots.

Evaluation of the type of cream aims to determine the type of emulsion from the cream preparation made. Tests carried out by the method of staining with methylene blue. The evaluation results of the type of cream are presented in the Table 7.

Table 7. Evaluation Results of Coffee Cream Types

Arabica Coffee			
	F1	F2	F3
R1	M/A	M/A	M/A
R2	M/A	M/A	M/A
Liberica Coffee			
	F1	F2	F3
R1	M/A	M/A	M/A
R2	M/A	M/A	M/A
Robusta Coffee			
	F1	F2	F3
R1	M/A	M/A	M/A
R2	M/A	M/A	M/A

In testing the methylene blue dripped on the cream preparation visually showed the results of the homogeneous distribution of the blue color in the cream preparation. So that the cream can be categorized into the type of oil in water (M / A).

Methylene blue or brilliant blue FCF has good solubility in water. So that it can be used as an indicator of the type of cream where if the cream preparation has a water dispersing phase (M / A) the positive results form a blue or greenish color which is easily spread evenly in the cream preparation.

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