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1.	**Sunscreen Factor Formulation and Test of Gel Preparations of 70% Ethanol Extract on Arabica Coffee Leaf (Coffea arabica L.)**

Demikian surat tugas ini diberikan kepada yang bersangkutan untuk dilaksanakan dengan penuh amanah dan tanggung jawab

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Sunscreen Factor Formulation and Test of Gel Preparations of 70% Ethanol Extract on Arabica Coffee Leaf (*Coffea arabica* L.)

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Sunscreen Factor Formulation and Test of Gel Preparations of 70% Ethanol Extract on Arabica Coffee Leaf (*Coffea arabica* L.)

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Abstract. Arabica coffee leaf extract (*Coffea arabica* L.) contains phenolic and flavonoid compounds that are potentially active as sunscreen because it might absorb UV (Ultra Violet) A and UV B rays. It is well-known that UV rays exposure causes a negative effect to skin, yet is possible to be avoided by applying sunscreen. This research aims to test the effect of arabica coffee leaf extract on formulation of gel preparations and to discover how its concentration affects the amount of gel preparations' sunscreen factor. There were 4 formulas with different extract concentrations whose characteristics were tested chemically, physically, and they were tested to measure its SPF number. From this test, it was discovered that the gel was qualified and it was plastic thixotropic flow. The measurement of SPF number proposed that F1 could not be categorized as protector because it was only 0.6 while F2, F3, and F4 showed that these formulas could be categorized as minimum protector. The samples were also tested statistically by using One Way ANOVA and Tukey HSD test. The result of the ANOVA test showed the number of sig 0.000 < 0.05 which meant all four formulas were significantly different. The research concludes that the extract of arabica coffee leaf might affect the formulation of gel preparations and its extract concentration might affect SPF number on gel preparations.

1. Introduction

Skin does an important role to protect the body from outside environments such as physical impact and exposure to free radicals. In order to maximize the function of the skin as a protector, it is necessary to give special treatment on the skin. Amongst information about how dangerous UV rays affect our skin, it is also well-known that sunscreen has been trusted to protect our skin from the damage of UV rays[1].

UV rays are rays that are emitted by the sun that can reach the earth surface beside visible light and infrared rays. The wavelength of UV rays is approximately 200-400 nm. The UV spectrum, based on the wavelength, is categorized into three; UV C (200-290), UV B (290-320) and UV A (320-400). Not all of these three UV rays' radiation can reach earth's surface. UV C rays, the one with the strongest energy, cannot reach the surface because of being absorbed in ozone [2].

It is known that UV radiation from the sun causes some damages to human skin. UV A radiation is discovered to cause damages to skin cells and DNA. It also causes photoaging and photocarcinogenesis. These damages can be caused by long term exposure of the sun's rays, even in a small dose. UV A radiation contributes to the decreasing of skin elasticity, the increasing of wrinkles, and the increasing of free radicals that cause acute to chronic changes to skin. Besides, UV A radiation

also makes other diseases worsen, such as lupus erythematosus. It also contributes as immunosuppressants that increase cancer cell growth [3]. From another resource, it is stated that the exposure of ultraviolet radiation might cause epidermis damages, which is also called sunburn, pigmentation, wrinkling, premature skin aging, and long term exposure of the sun's rays can also cause mutation of lugature tissue in the stratum corneum[4]

On the other hand, the sun's rays stimulate melanin and pigment as natural sunscreen for human skin. The sun's rays also stimulate hormone protection which allows vitamin D synthesizing to increase skin cell regeneration. It is discovered that the shorter the wavelength, the stronger the energy level, which also causes more damage to the skin (Lu et al., 1996). If the sun's rays expose the skin excessively, it might not resist the negative effects. Therefore, it is necessary to give some protection by using sunscreen. Sunscreen is preparations applied on skin to absorb, to scatter, or to reflect UV rays. It is also applied to help natural protection mechanisms to protect skin from dangerous UV radiation of the sun's rays [6]. It is a product that provides protection from the damage of the sun's rays, but this product is commonly made of synthetic chemicals which allow, in long term exposure, some bad effects to human skin. This research is conducted, to test the formula, so that there would be a chance for natural ingredients to be natural protection which causes less bad effect [7]. The grade of sunscreen's photo-protective is universally measured by Sun Protective Factor (SPF) which determines the increase of the sun's rays' exposure dose with photo-protective products applied without eritema [8].

Coffee is categorized into genus Coffea in the family of Rubiaceae. Genus Coffea L. consists of more than 100 species, but only two species, *Coffea arabica* (arabica coffee) and *Coffea canephora* (robusta coffee), are commercially cultivated on large scales [9]. *Coffea arabica* is a tetraploid species from highland rain forest in the south of Ethiopia. Six of eight chosen species are closely related, including two commercial species *Coffea arabica* dan *Coffea canephora*, as illustrated in the recent phylogeny from genus [10].

Coffee leaf has not been optimally utilized. In Indonesia, coffee leaf is used as a brewed drink named "Aia Kawa" in Sumatera [11]. It is discovered that *Coffea arabica* leaf contains phenolic compounds with a concentration of 17.4% in green leaf and 13.9% in old/pure leaf (Rodrigues et al., 2008). Arabica coffee leaf contains some compounds, such as alkaloid, flavonoid, and phenol which are included into the derivative of hydroxycinnamic acid, caffeine, chlorogenic acid, coumarin, ferulic acid and cinnamic acid. Chlorogenic acid is easily oxidized in aqueous solutions [13] [14].

Phenolic and flavonoid compounds have potential as sunscreen [15]. Flavonoid is commonly able to absorb UV rays, but not able to absorb any radiation on wavelength around 280-315 nm maximally, as other phenylpropanoid compounds. However, compared with other phenylpropanoid compounds, flavonoid compounds will increase significantly, as it is exposed by UV B rays' radiation, including the derivative of hydroxycinnamic acid (for instance: p-Coumaric acid, ferulic acid and caffeine acid). Some research showed that when UV B rays' exposes plants, flavonoid concentration is increased compared with hydroxycinnamic compounds [16].

Sunscreen is applied to help natural protection mechanisms to protect skin from dangerous UV radiation of the sun's rays [6]. It is a product that provides protection from the damage of the sun's rays, but this product is commonly made of synthetic chemicals. In the market, sunscreen is provided in some forms of preparations; salve, cream, gel, lotion, spray, and wax stick [17]. Gel is a semi-solid preparation which consists of suspensions made of inorganic particles in small parts or organic molecules in big parts penetrated by some kinds of fluid [18].

In Indonesia, research related to arabica coffee leaves is limited. Therefore, it is necessary to conduct further research because the resources are overflowing and it should be utilized and developed, for instance, for cosmetic purposes. This research aims to fill the void in the literatures related to arabica coffee leaf by testing how the extract of arabica coffee leaf affects the formulation of gel preparations and discovering how far the extract of arabica coffee leaf concentration affects the grade of Sun Protective Factor of gel preparations.

2. Material and Methods

2.1 Material

The materials used in this study were arabica coffee leaves (Coffea arabica L.) as the active ingredient which was obtained by maceration using 70% ethanol. The chemicals used for the gel formulation were HPMC K100M type 2208 (High Viscosity Grade), Propylene Glycol (Pharmaceutical Grade), Methyl Paraben (Pharmaceutical Grade), Propyl Paraben (Pharmaceutical Grade), Aqua Dest, 70% Ethanol and 70% Ethanol for analysis.

2.2 Plant Determination

The determination of the arabica coffee plant used in the research was carried out at the Herbarium Bogoriense, Botany Division, Biological Research Center, LIPI Cibinong.

2.3 Arabica Coffee Leaf Extraction

Coarse powder of arabica coffee leaves that have been obtained from IPB Bogor was re-pollinated using a grinder or blender. It was then sieved using a mesh sieve no. 40. The extraction method used maceration. The maceration extraction method (cold method) is a better method for extracting cherry leaves compared to the soxhlation method (hot method). This is due to the thermolable nature of flavonoids which will be damaged when extracted using a soxhletation [19]. 1:10 fine powder and macerated solvent. A total of 1300 grams of powder was macerated with 9700 ml of 70% ethanol filter in a dark glass jar at room temperature. It was then left for 2 days and stirred every 8 hours. The maceration results were filtered with a flannel cloth and filter paper. Then, the remaining dregs were re-macerated with 3300 ml of sprinkler for 1 day. Afterwards, it was stirred and filtered again. The filtrate obtained was then concentrated using a rotary vacuum evaporator at a temperature of 50 ° C until a thick extract was obtained. Thick extract of arabica coffee leaves, and the yield was obtained. The yield was calculated based on the ratio of the final weight (weight of the extract produced) to the initial weight (weight of cell biomass used) multiplied by 100% [20].

2.4 Organoleptic Determination of Arabica Coffee Leaf Extract and Determination of Ash Content

Organoleptic determination of arabica coffee leaf extract was carried out by observing the shape, color, and odor of the arabica coffee leaf extract. 2 g of extract was weighed carefully and put in a porcelain crucible that had been annealed,tared, and leveled. Krus was slowly annealed until the charcoal ran out. Afterglow was carried out at a temperature of 600ºC for 3 hours, which was then cooled and weighed until a fixed weight was obtained. The ash content was calculated against the material which was dried in the air. If in this way the charcoal cannot be removed, hot water can be added and filtered with an ash-free filter paper. The rest of the paper and filter paper was glazed in the same crucible. The filtrate was put into the crucible, and left to evaporate. Afterglow until the weight was fixed, weighed and calculated.

2.5 Phytochemical Screening Test

Extract was identified of alkaloid, flavonoid, phenol, saponin, tannin, steroid, and triterpenoid contents using Harbonne or Material Medica Method.

2.6 Gel Formulation

Arabica coffee leaf extract gel formula (*Coffea arabica* L.) consisted of arabica coffee leaf extract (*Coffea arabica* L.), HPMC K100M type 2208, Propylene Glycol, Methyl Paraben, Propyl Paraben and Aqua Dest. The gel formulation can be seen in table (1).

Table 1. Formulation Gel Arabica Coffee Leaf Extract

No.	Materials	% (b/v)				Functions
		F1	F2	F3	F4	
1	Ekstract	-	1.5	2	2.5	Active ingredients
2	HPMC	1	1	1	1	*Gelling agent*
3	Propylene Glycol	15	15	15	15	Humectant
4	MethylParaben	0.075	0.075	0.075	0.075	Preservatives
5	Propylparaben	0.025	0.025	0.025	0.025	Preservatives
6	Aqua Dest ad	100	100	100	100	solvent

2.7 Procedure for Preparation Arabica Coffee Leaf Extract Gel

HPMC 4.5 g was developed in a mortar with aqua dest and was then expanded for 24 hours and crushed to form a gel mass (M1). 0.3775 g of methyl paraben was dissolved with a portion of propylene glycol (M2). 0.1125 g of propyl paraben was dissolved with a portion of propylene glycol (M3). The arabica coffee leaf extract was also dissolved with the remaining propylene glycol (M4). M1, M2 and M3, M4 were mixed, crushed, and homogeneous. Furthermore, the evaluation of the determination of the FPS value of gel preparations were carried out.

2.8 Organoleptic Test for Gel

Organoleptic testing was carried out by direct observation of the shape, color and smell of the gel made. Gel was usually clear with a semi-solid consistency[18].

2.9 Homogeneity Test

The homogeneity test was carried out by applying 0.1 g of gel preparation to a piece of transparent glass and observing its homogeneity. The test preparation must show a homogeneous arrangement, indicated by the absence of coarse grains on the object glass [21].

2.10 pH test

The pH test was carried out by turning on the pH meter then dipping the pH meter electrode into the gel formula. It was left for a few moments until the pH meter screen showed a stable number [22].

2.11 Spreadability Test

As much as 1 gram of gel preparation was placed carefully on a glass measuring 20x20 cm, and was given a 125 gram weight on it.It was then measured the diameter formed after 1 minute [23].

2.12 Adhesion Test

A total of 1 gram of gel preparation was flattened on one glass object and covered with another glass object until the two plates were joined. The object glass pair was pressed with a load of 1000 g for 5 minutes. It was then installed on the adhesion test equipment, while simultaneously recording the time it took for the two plates to come off each other [24].

2.13 Viscosity and Flow Properties Test

The viscosity test was performed using the Brookfield Viscometer RV type. The gel preparation was put into a 500 mL beaker glass container, then 'the spindle no. 04 was installed. Afterwards, the spindle was lowered into the preparation to the specified limit. The tool was turned on and set at a speed

of 10 rpm, 12 rpm, 20 rpm, 30 rpm and 50 rpm to show a stable number. Then, the results were recorded. To determine the flow properties of the preparation, a curve was made between the shear rate and shear stress [21].

2.14 Centrifugation Test

10 grams of each preparation were put into a centrifugation tube and centrifuged at 3750 rpm for 5 hours. It was then observed whether there was separation or not in the gel [21].

2.15 Freeze Thaw Test

The phase separation cycle using the freeze thaw method on the gel preparation was carried out in 6 cycles for each formula. Each cycle was observed after 48 hours of storage at 4°C and 48 hours after at 45°C for 24 days. Each cycle was observed whether there was phase separation or not on the gel preparation [21].

2.16 Determination of the FPS Value of Arabica Coffee Leaf Extract

The FPS value of the extract in vitro was determined using the Uv-Vis spectrophotometric method developed by Mansur (1986) using equation 3. The extract was diluted with a concentration of 60 ppm, 80 ppm and 100 ppm with 70% pa ethanol and then measured using the Uv-Vis spectrophotometer at a wavelength of 290-320 nm. The determination of the FPS value was carried out in three replications. Then, the data obtained was processed with the Mansur equation (1986). To determine the FPS value of this wavelength, the EE x I value has been described in table 2 [7].

2.17 Determination of the FPS Value of Arabica Coffee Leaf Extract Gel Preparation

The FPS value of the extract in vitro was determined using the Uv-Vis spectrophotometric method developed by Mansur (1986) using equation 3. The preparation was weighed as much as 0.02 grams in 5 mL ethanol 70% pa and then measured using a Uv-Vis spectrophotometer at a wavelength 290-320 nm. The determination of the FPS value was carried out in three replications. Then, the data obtained was processed with the Mansur equation (1986). To determine the FPS value of this wavelength, the EE x I value has been described in table 2 [7].

The equation for determining the FPS value can be seen in below:

$$SPF = CF \; x \sum_{290}^{320} \; x \; EE(\lambda) \; x \; I(\lambda) \; x \; Abs(\lambda)$$

Based on this equation, there was a variable CF (Correction Factor) = 10, EE (Erythemogenic Effect), I is the simulated intensity of sunlight and Abs is the absorbance of the sample.

The value of EE x I can be seen in table 2.

Table 2. The Value of EE x I

Wave Length (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

According to the FDA (Food Drug Administration), the distribution of sunscreen capabilities is Minimum (SPF is between 2-4), Medium (SPF is between 4-6), Extra (SPF is between 6-8), Maximum (SPF is between 8-15), and Ultra (SPF is more than 15) [25].

2.18 Statistical Analysis

Qualitative testing of physical characteristics and determination of the FPS value of gel preparations were analyzed using One Way ANOVA, with a confidence level of 95% ($p > 0.05$). If there WEre significant differences between formulas, we then proceeded with the Tukey HSD test to see which formulas had significant differences.

3. Results and Discussion

The object of this research was fresh arabica coffee plants (*Coffea arabica* L.). Then, the fresh arabica coffee plants were determined in Herbarium Bogoriense, Botany Department of Biology Research Center, LIPI Cibinong. The determination of the plants in this research meant to find out the clear identity of the research object to avoid mistakes in collecting main material for the research. After determined, it was confirmed that the plants were arabica coffee leaf (*Coffea arabica* L.).

Table 3. The Results of Characterization pf Arabica Coffee Leaf Extract

Examination	Results
yield	9.84%
Organoleptic:	
a. form	Thick extract
b. smell	coffee
c. taste	bitter
d. color	Chocolate black
Drying shrinkage	6.61% ± 0.43
Ash content	9.26% ± 0.46

Based on the results of phytochemical screening tests, 70% thick ethanol extract of arabica coffee leaves had alkaloid, phenolic, flavonoid, saponin, tannin and steroid compounds. This is in accordance with previous research [26], [27]. By knowing the truth of its benefits, arabica coffee leaf extract can be formulated into gel preparations that contain antioxidants.

Table 4. Results of Phytochemical Screening Test

Compound	Result
Phenolic	+
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	+
Steroids	+
Triterpenoid	-

Organoleptic tests included observations on the consistency, color and odor of the gel preparation. The results of organoleptic testing can be seen in table 5. In accordance with the results from the organoleptic test, it was found that all four formulas had semi-solid consistency. For F1, the gel was clear/transparent and odorless because it was not mixed with the extract, while for F2, F3, and F4, they were clear brown and had a typical smell from the extract. The extract concentration affected the color of gel preparations, the higher the concentration, the deeper the brown color.

Homogeneity test aimed to determine the homogeneity of preparations that have been made, homogeneous preparations will produce good quality because it showed the active ingredients of the drug are evenly dispersed in the basic ingredients. Based on the research results, the four formulas produced a homogeneous gel.

Table 5. Results of Organoleptic Tests and Homogeneity Tests

Formula	Organoleptic			
	Consistency	Color	Smell	Homogeneity
1	Semisolid	Transparent	Odorless	Homogeneous
2	Semisolid	Clear brown	Unique	Homogeneous
3	Semisolid	Clear brown	Unique	Homogeneous
4	Semisolid	Clear brown	Unique	Homogeneous

The purpose of this test was to determine that the resulting gel was acceptable for skin pH, because if it is not in accordance with the pH of the skin it can cause irritation. Based on the results of the study, the four formulas met the pH criteria on sunscreen preparations. pH test discovered that all formulas were qualified for pH criterias on sunscreen preparations, which was around 4.5-8.0 (SNI, 1996). The extract concentration affected the acid of the preparations because the extract contained a flavonoid compound which was quite acidic. Therefore, the more the arabica coffee leaf extract concentration, the more the acid it has (Markham, 1988). Arabica coffee leaf extract was 6.16 pH. A product that had too high or too low pH could cause irritation on skin [6]. The graph of the pH test results can be seen in Figure 1.

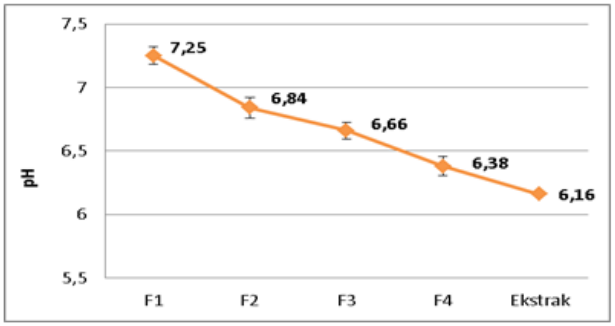

Figure 1. The Results of pH test

The dispersion test was carried out to ensure that the semisolid preparations are able to spread easily without significant pressure, so that they are easy to apply without causing pain and provide comfort to the user. The graph of the results of the scattering power test can be seen in Figure 2. The greater the spreadability of the preparation, the greater the ability of the active substance to spread and contact with the skin. The spreadability is inversely proportional to viscosity, the greater the viscosity of a preparation, the thicker the consistency, so that the resulting spreadability is smaller [30].

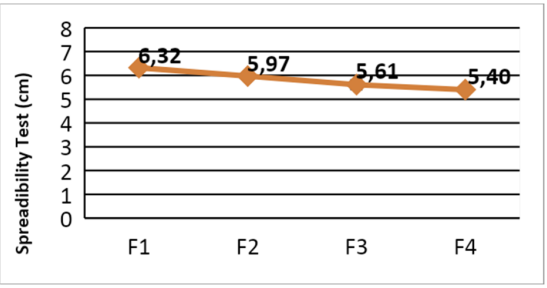

Figure 2. The Results of Spreadability Test

The adhesion test was carried out to determine the ability of the gel to adhere to the skin surface. The graph of the adhesion test results can be seen in Figure 3. Based on the research results, the four formulas met the adhesion criteria. Topical preparations must have sufficient adhesiveness but should not be sticky to the skin as they can reduce comfort during use.

The viscosity test was aimed to determine the thickness of the gel preparation. The higher the viscosity value, the higher the viscosity level of the gel preparation. Viscosity was tested by using *Brookfield* Viscometer RV type. The result revealed that all formulas were qualified for criterias of gel viscosity, which was 2000-4000 *cPs.* [31]. Gel consistency, which was not too liquid or too thick, was a characteristic of the good gel. HPMC concentration in gel preparations was used only 1%, as HPMC K100M is categorized into *High Viscosity Grade* which can be read in *Certificate of Analysis*. Gel viscosity affects the gel itself to disperse and adhere to skin. The higher the viscosity, the more difficult the gel to disperse although it is easier to adhere on skin. The higher the viscosity, the smaller the separation rate of dispersed phase [32]. Of the four formulas, the gel viscosity criteria were met. The graph of the results of the viscosity test can be seen in Figure 4.

All four formulas produced plastic thixotropic flow. It could be seen that the curve was started without getting through point zero (0) but cutting the axis of motion stress (or the outside of the curve was extrapolated cutting the axis) at the certain points named yield grade [33]. It was also shown that the decreased curve was to the left of the increased curve because there was structural change that did not immediately return to the first condition, if the pressure was diminished. Yield grade resulted from F1 was f = 3.496 dyne/cm², F2 was f = 3.097 dyne/cm², F3 was f = 2.979 dyne/cm² and F4 was f = 3.557 dyne/cm². Thixotropic is the characteristic of the flow that is expected for semi-solid preparations because it is expected to be in high consistency in a container. Nevertheless, it is easier to pour when it is given force, making it easier to be applied on skin [34]

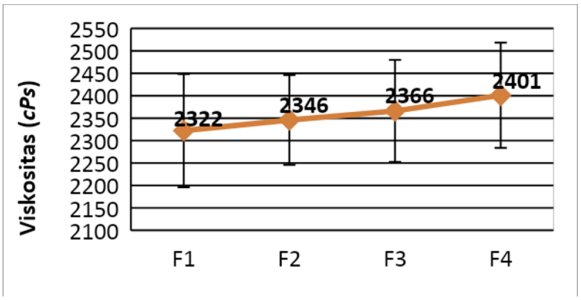

Figure 3. The Results of Viscosity Test

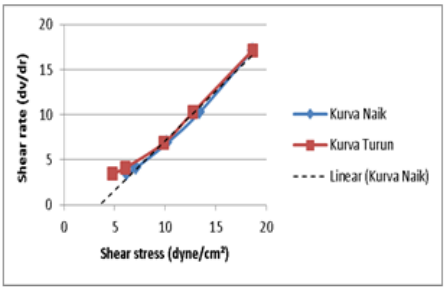

Figure 4. Flow Properties Formula 1

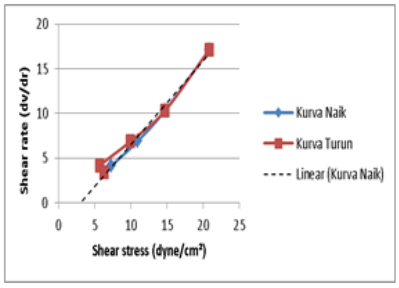

Figure 5. Flow Properties Formula 2

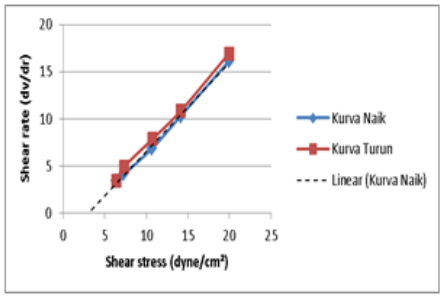

Figure 6. Flow Properties Formula 3

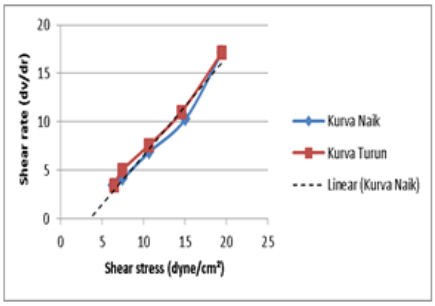

Figure 7. Flow Properties Formula 4

The centrifugation test aimed to observe whether there is a phase separation of the gel preparation and to see the stability of the gel preparation after very strong shaking. Based on the research results, the four formulas did not undergo phase separation or syneresis. The table of centrifugation test results can be seen in table 6.

Table 6. Centrifugation Test

Formula		Results
F1	1	-
	2	-
	3	-
F2	1	-
	2	-
	3	-
F3	1	-
	2	-
	3	-
F4	1	-
	2	-
	3	-

Note : (-) no separation phase

The freeze thaw test is a test that has the purpose of simulating the product during the distribution process in vehicles, which are rarely equipped with temperature control devices. Therefore, this test was carried out at a certain temperature or humidity at a certain time so that the product in its packaging will undergo various changes. If during the six cycles, the preparation is stable or there is no phase that changes, it means that the product is stable during the distribution process [35]. Based on the results of the freeze thaw test, the four formulas were stable as there was no phase separation. The table of freeze thaw test results can be seen in table 7.

Table 7. Results of *Freeze Thaw*

	cycle	F1			F2			F3			F4		
		1	2	3	1	2	3	1	2	3	1	2	3
1	4°C	-	-	-	-	-	-	-	-	-	-	-	-
	45°C	-	-	-	-	-	-	-	-	-	-	-	-
2	4°C	-	-	-	-	-	-	-	-	-	-	-	-
	45°C	-	-	-	-	-	-	-	-	-	-	-	-
3	4°C	-	-	-	-	-	-	-	-	-	-	-	-
	45°C	-	-	-	-	-	-	-	-	-	-	-	-
4	4°C	-	-	-	-	-	-	-	-	-	-	-	-
	45°C	-	-	-	-	-	-	-	-	-	-	-	-
5	4°C	-	-	-	-	-	-	-	-	-	-	-	-
	45°C	-	-	-	-	-	-	-	-	-	-	-	-
6	4°C	-	-	-	-	-	-	-	-	-	-	-	-
	45°C	-	-	-	-	-	-	-	-	-	-	-	-

Note: (-) no separation phase

The Sun Protection Factor (SPF) value or FPS value is measured as the ability or effectiveness of a material as a sunscreen. The higher the FPS value, the better the sunscreen will protect against UV rays. The purpose of using sunscreen is based on its ability to absorb, reflect or scatter sunlight [36]. The value of FPS was determined in vitro using the UV-vis spectrophotometry method developed by Mansur (1986). It was done by measuring the absorbance of each sample, namely the extract of arabica coffee leaves and the four formulas for the gel preparation for three replications with a wavelength between 290 - 320 nm where the measurements were described for each 5 nm interval. It is known that the arabica coffee leaf extract has these groups found in flavonoids and phenolic compounds, making it able to absorb radiation energy and produce absorbance values on the uv-vis spectrophotometer.

Based on the results of the study, the arabica coffee leaf extract has a concentration of 60 ppm. Of the three extract concentrations, it has a minimal protection category. The F1 gel preparation was a negative control, which did not contain extracts but produced a low FPS value for sunscreen so that it did not fall into the protection category. F2, F3 and F4 have a minimal protection category. The results of determining the FPS value of extracts and gel preparations can be seen in table 8 and table 9. From the second to fourth formulas, all formulas had minimal protection categories. From the results, it was obtained that gel preparations produce low protection on the skin. This can be overcome by adding other active sunscreen ingredients to support the effectiveness of sunscreens to produce more maximally.

On the SPF grade of the gel preparation that was higher than the extract, from the results of the research of [38] Donglikar and Deore (2017), it was revealed that the phytochemical SPF of the formulation was higher, which indicated the synergy and compatibility of the excipients as well. This result indicated that the prepared formulation has good SPF and good sun protection activity. Moreo et al., (2013) explained that this is possible to happen because it is influenced by the combination and concentration of the gel carrier component, the type of gel, the effect and the interaction of the carrier component such as the humectant used in the formulation. This factor can increase or decrease the UV absorption of sunscreens [39].

Therefore, to develop sunscreens with better safety and high SPF, the formulator must understand the physicochemical principles of not only active UV absorbance, but also vehicle components, such as esters, emulsifiers and emulsifiers used in formulationsThis is because sunscreens can interact with other components of the vehicle, and this interaction can affect the efficacy of the sunscreen.

Table 8. Results of SPF Value Extract

Sample	FPS Va;ue	Protection Category
Ekstract 60 ppm	2.08	Minimum protection
Ekstract 80 ppm	2.49	Minimum protection
Ekstract 100 ppm	3.68	Minimum protection

Table 9. Results of SPF Value Gel Extract

Sample	Value SPF	Protection Category
Formula 1	0.61	-
Formula 2	2.59	Minimum protection
Formula 3	2.95	Minimum protection
Formula 4	3.64	Minimum protection

4. Conclusions

Based on the research results, arabica coffee leaf extract could affect the gel dosage formulation and meet the specified requirements. The results of the ANOVA test showed a grade significance of 0.000 < 0.05, where there were significant differences in the four formulas for gel preparations. This showed that the concentration of arabica coffee leaf extract could affect the SPF value of the gel preparation. In this study, a gel preparation formulation that met the pharmaceutical requirements was obtained by providing an SPF grade with minimal protection.

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1.	**Antioxidant Activity and Sun Protection Factor (SPF) Graded Extract of Katuk Leaves (Sauropus androgynus (L.) Merr.)**

Demikian surat tugas ini diberikan kepada yang bersangkutan untuk dilaksanakan dengan penuh amanah dan tanggung jawab

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Antioxidant Activity and Sun Protection Factor (SPF) Graded Extract of Katuk Leaves (*Sauropus androgynus* (L.) Merr.)

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Abstract. Katuk leaf (*Sauropus androgynus* (L.) Merr.) is believed to have medicinal properties, one of which is as an antioxidant. Its efficacy as an antioxidant cannot be separated from the phenolic and flavonoid compounds contained in katuk leaves. This study aims to determine the total phenolic and flavonoid levels as well as the antioxidant activity of graded extracts, namely *n*-hexane, ethyl acetate, and 70% ethanol extract of katuk leaves. The results showed that the total phenolic content and total flavonoid content of each extract were significantly different. Antioxidant activity by the DPPH method was calculated at IC_{50} using quercetin as a comparison. The highest antioxidant potential was shown in the ethanol extract. Each extract has the potential as a sunscreen and ethanol extract provides the highest SPF value.

1. Introduction

Phenol compounds are characterized by the presence of an aromatic ring and one or two hydroxyl groups. Phenol compounds that have more than two hydroxyl groups are called polyphenols, for example the tannin, flavonoid, melanin, and lignin groups [1]. Flavonoid compounds have a characteristic structure of two aromatic rings connected by three C atoms, usually with O atomic bonds in the form of heterocyclic oxygen bonds [1]. Flavonoids are a group of secondary metabolites produced by plants which are included in the large group of polyphenols. Flavonoids have the ability to scavenge free radicals and inhibit lipid oxidation [2].

Sunscreen contains compounds that can protect the skin by absorbing ultraviolet (UV) rays emitted by the sun [3]. Compounds contained in sunscreen can be used to prevent various skin diseases and to protect human skin health from the negative effects of UV rays. Sunscreens are divided into 2 groups, namely physical sunscreens and chemical sunscreens. Chemical sunscreens are sunscreens that absorb ultraviolet light, such as PABA, PABA esters, benzophenone, avobenzone, salicylate, cinnamate and camphor derivatives [4].

Katuk leaves (*Sauropus androgynus* (L.) Merr.) contain secondary metabolites such as tannins, saponins, alkaloids, flavonoids, glycosides, and phenols [5]. The 95% ethanol extract of katuk leaves has a high content of phenolic compounds with 1.49 mgGAE/g fresh weight and 8.71 mgGAE/g dry weight [6]. Antioxidant activity in previous studies stated that the IC_{50} value of katuk leaf methanol extract had a value of 80.81 ppm [7]. Variations in ethanol concentration and extraction methods resulted in different phenolic and flavonoid content in katuk leaves [8], [9]. Multilevel extraction gives better results than individual extraction with each solvent [10].

The aim of the study was to determine the levels of phenolic, flavonoid, as well as antioxidant activity and SPF testing of the *n*-hexane, ethyl acetate, and 70% ethanol extract of katuk leaves from the extraction process with graded maceration, using UV-Vis spectrophotometry method.

2. Materials and Methods

2.1 Preparation Extract

Katuk (*Sauropus androgynus* (L) Merr) leaves were collected and determined at the Institut Pertanian, Bogor, West Java. Simplicia processing begins with fresh katuk leaves taken and cleaned of impurities, then washed with water until clean, drained and finely chopped, and then dried again by aerating. After dried then powdered.

The graded extract was obtained by a stepwise method using solvents with different polarity levels, namely *n*-hexane, ethyl acetate, and 70% ethanol (1:10). The material is soaked for 24 hours, stirring occasionally for the first 6 hours. The residue is then separated from the filtrate. The process is repeated until the solvent is clear (3 repetitions). The residue was then extracted with ethyl acetate and 70% ethanol using the same procedure. Each maserate obtained was then concentrated with a vacuum rotary evaporator at a temperature of 40ºC - 50ºC to obtain a thick extract.

2.2 Characterization of Extract

The organoleptic test was carried out by observing the shape, color, smell, and taste using the five senses. Each extract was weighed carefully as much as 2 grams, then put into a silicate crucible that had been sized and compared, incandescent in a kiln and the temperature was gradually increased to 600°C (temperature difference of approximately 25°C) until carbon-free. Next, it was cooled in a desiccator and then weighed. The total ash content is calculated against the weight of the test material and is expressed in % w/w. Each 1.0 gram extract was placed in a moisture content balance container which had been previously sized and the initial weight of the extract was seen. Then the extract was dried at a temperature of 105°C at the moisture content balance until a constant extract weight value was obtained, afterward the final weight of the extract was seen. Results are viewed as % MC values [11].

2.3 Phytochemical Screening of Extract

The phytochemical compounds of kencur (aromatic ginger-*Kaempferia galanga*) ethanolic extract, such as phenolic, flavonoid, tannin, alkaloid, and terpene were qualitatively identified following standard procedures describing in the Harborne and Indonesian Herb Pharmacopoeia [12].

2.4 Determination of Total Phenolic Level

From the base solution of 100 ppm gallic acid, several concentrations were made, namely 18 ppm, 30 ppm, 42 ppm, 54 ppm, and 66 ppm. From each concentration of the standard solution of gallic acid, 300 µl was pipetted and then 1.5 ml of *Folin-Ciocalteu* reagent (1:10) was added. After being allowed to stand for 3 minutes, each solution was added with 1.2 ml of 7.5% Na_2CO_3 solution, shaken homogeneously, and allowed to stand in the operating time range at room temperature. All solutions were measured for absorbance at the maximum absorbance wavelength obtained, which was 756.5 nm, then a calibration curve was made for the relationship between gallic acid concentrations. The extract solution obtained was pipette 300 l and added 1.5 ml of *Folin-Ciocalteu* reagent and shaken. It was allowed to stand for 3 minutes, added 1.2 ml of 7.5% Na_2CO_3 solution and allowed to stand again in the operating time range at room temperature. The absorbance of the extract solution was measured by UV-Vis spectrophotometer at the maximum absorbance wavelength. The absorbance of the extract solution was measured by UV-Vis spectrophotometer at a wavelength of 756.5 nm. Performed 3 repetitions [13].

2.5 Determination of Total Flavonoid Level

From 1000 ppm quercetin mother liquor, several concentrations were made, namely 33, 57, 81 and 105 ppm. A total of 0.5 ml of quercetin solution was made with several concentrations of pipette then added with 1.5 ml of methanol and added 0.1 ml of 10% $AlCl_3$ reagent 0.1 ml of sodium acetate (1M) and 2.8 ml of distilled water. Then the solution was shaken and left for an operating time of 60 minutes at room temperature. Measure the absorbance at a wavelength of 434 nm against the standard. Each concentration of 0.5 ml of the test solution was added with 1.5 ml of methanol and added 0.1 ml of 10% $AlCl_3$ reagent 0.1 ml of sodium acetate (1M) and 2.8 ml of distilled water, then allowed to react during the operating time at room temperature. Measure the absorption at the maximum wavelength against the standard [14].

2.6 Antioxidant Activity Test

Weigh 100.0 mg of ethanol extract of katuk leaves from each extraction time dissolved with methanol in a volumetric flask, then diluted to obtain concentrations of 20, 40, 60, 80 and 100 ppm. A total of 0.2 mL of sample solution of each concentration was added with 1 mL of 0.5 mM DPPH and 5 mL of methanol, allowed to stand according to the operating time for 30 minutes and then measured at a maximum wavelength of 515.5 nm. The percentage of radical scavenging activity was calculated by the formula [15].

2.7 Sunscreen Potency Test

The ethanol extract of Arabica coffee leaves was diluted with ethanol to a concentration of 100 ppm. The SPF value was determined by measuring the absorbance of the solution from the extract using a UV-Vis spectrophotometer at a wavelength of 290-320 nm. The sample was dissolved in 5 mL of ethanol p.a. The determination of the SPF value was carried out three times for each sample. The absorbance data obtained were processed by the equation of Mansur (1986) [16].

$SPF = CF \times EE \times I(\lambda) \times abs(\lambda)$

The SPF value can be calculated by multiplying the correction factor (CF), the erythema effect spectrum (EE), the intensity spectrum from the sun (I), and also the absorbance (Abs) of the cream sample of coffee leaf ethanol extract. The SPF assessment refers to the provisions of the FDA (Food and Drug Administration), namely minimal protection if SPF 2-4, moderate protection 4-6, extra protection 6-8, maximum protection 8-15, and ultra protection >15 [17].

2.8 Data Analysis

The observed data from the dispersion test and color stability test were then analyzed using one-way ANOVA. If there is a significant difference between the formulas, then it is continued with the Tukey HSD test with a 95% confidence level to see if the formula has a significant difference or not.

3. Results and Discussion

The purpose of using three solvents with different polarities is to obtain active compounds from katuk leaves based on their polarity [10]. In this study, repetition was carried out with the aim of providing sufficient extract weight and being able to attract residual compounds that were still left in the previous iteration. Extraction using solvents with different polarities will produce different polyphenol components so that the antioxidant properties or characteristics of each compound obtained from the extraction are also different [18].

Table 1. Katuk Leaf Extraction Results

Extract type	Average extract weight (g) ± SD	Average extract yield (%) ± SD
n-Hexane	18,05 ± 0,73	7,22 ± 0,29
Ethyl acetate	14,87 ± 1,15	5,95 ± 0,46
Ethanol 70 %	50,27 ± 1,90	20,11 ± 0,76

Table 2. Characteristics of Katuk Leaf Extract

Parameters	Extract type		
	n-Hexane	Ethyl acetate	Ethanol 70%
Organoleptic			
Form	Thick extract	Thick extract	Thick extract
Smell	Typical	Typical	Typical
Favor	Bitter	Bitter	Rather Bitter
Color	Green	Green	Chocolate
Ash Level	2,59 % ± 0,22	0,55 % ± 0,05	6,88 % ± 0,93
Drying Shrinkage	1,08 % ± 0,48	3,04 ± 0,58	3,48 ± 2,41

The yield (Table 1) of the extract obtained from the multilevel extraction resulted in the lowest yield of the ethyl acetate extract, followed by the *n*-hexane extract. The yield obtained in the multistage extraction with 70% ethanol showed the highest yield. Based on the principle of mass transfer of solute into a solvent that is in accordance with characteristics *like dissolves like*, where there is a transfer at the interface layer (solvent and solute) then the solute diffuses into the solvent. The results are different because the ability to attract compounds in each solvent is different too. 70% ethanol extract has the highest yield because 70% ethanol solvent is universal. It can attract non-polar and polar compounds so that 70% ethanol extract is attracted to more secondary metabolites [8].

Organoleptic test on leaf extract was conducted to determine the characteristics of shape, smell, taste, and color. Of the three katuk leaf extracts the difference was only in the extract, the *n*-hexane, and ethyl acetate extracts had a green color while the 70% ethanol had a brown color.

The ash content obtained from this research is *n*-hexane 2.59%, ethyl acetate 0.55%, and ethanol 70% 6.88% as seen in (Table 2). The three extracts had simplicia criteria that were in accordance with the guidelines for a good extract ash content, namely the ash content of not more than 12%. The purpose of ash content is to show the content of organic and inorganic compounds from plants such as alkali metals, alkaline earth metals and heavy metals, as well as to provide an overview of internal and external mineral content until they become extracts.

Drying shrinkage has a goal to give the maximum amount of compounds lost in the drying process. In this study, a moisture balance was used at a temperature of 105ºC from the three extracts of *n*-hexane, ethyl acetate, and 70% ethanol. The three results (Table 2) fall into the drying shrinkage limit of no more than 10%.

Table 3. Phytochemical Screening

Detected compounds	Extract type		
	n-Hexane	Ethyl acetate	Ethanol 70%
Alkaloids			
- Dragendor	-	-	+
- Bouchardat	-	-	+
- Mayer	+	+	+
Phenolic	+	+	+
Flavonoids	+	+	+
Saponins	+	+	+
Tannins	+	+	+
Terpenoids	-	-	-
Steroids	+	+	+

Note: (+) There is a compound (-) There is no compound

The results of phytochemical screening can be seen in (Table 3). Phytochemical screening was carried out to identify the compounds contained in *n*-hexane extract, ethyl acetate extract, and 70% ethanol extract of katuk leaves. Screening is an important step in an effort to reveal the potential of plant resources [1]. The 70% ethanol extract showed positive results for alkaloids, while the ethyl acetate and *n*-hexane extracts were negative.

Determination of total phenolic levels uses the *Folin-Ciocalteu* method because it is easy, cheap, fast to do, and can be done routinely in the laboratory. Polyphenols in plant extracts react with the reagent used, namely *Folin-Ciocalteu* (Blainski et al., 2013). Phenolic compounds react with *Folin-Ciocalteu* reagent only in an alkaline environment so that proton dissociation occurs in phenolic compounds into phenolic ions, hence 7.5% Na_2CO_3 is used to make an alkaline environment. The hydroxyl group in the phenolic compound reacts with the *Folin-Ciocalteu* reagent to form a blue *molybdenum-tungsten* complex which can be detected by a spectrophotometer [19]. Gallic acid (3,4,5-*Trihydroxybenzoic acid*) was used as standard. Gallic acid was chosen as the standard because it represents the general properties of phenolic compounds. In this case gallic acid is a natural phenolic compound that has a strong antioxidant effect [20].

Determination of the maximum wavelength of gallic acid aims to determine at what wavelength the compound reaches the highest absorbance value. The result of measuring the maximum wavelength is 765.50 nm with an absorbance of 0.3445 as the maximum absorbance value. The operating time of the results obtained is stable absorbance at 60 minutes so that it can be concluded that the purpose of determining the operating time is to get the measurement time when the reaction has been running optimally which is characterized by a stable absorbance, so as to maximize the measurement [21].

Various concentrations of gallic acid standards were made because the assay used a calibration curve equation (Table 4), so to make a calibration curve, several concentrations were made in order to obtain a linear equation. The results obtained were at concentrations of 18, 30, 42, 54, and 66 ppm, the data met the requirements of *Lambert Beer*'s law. The calibration curve obtained by the linear regression equation $y = 0.0093.x + 0.1824$ with a correlation coefficient (r) = 0.9944.

In the determination of the total phenolic content of katuk leaf extract, as seen in (Table 5) that the 70% ethanol extract had the highest concentration of 16.71 mgGAE/g. The extract was followed by ethyl acetate and *n*-hexane with a concentration of 2.95 mgGAE/g and 0.57 mgGAE/g extract, respectively. Phenolic compounds are extracted well in 70% ethanol because the phenolic content will increase in the extract as the polarity of the solvent increases. Phenolic also tends to be polar so it can dissolve in polar solvents well. These results are similar to other studies which stated polar extracts have the highest phenolic content followed by semi-polar and non-polar extracts. Methanol, a polar solvent, has a higher phenolic content than acetone which is a semi polar solvent [22].

Table 4. Results of Total Phenolic Levels

Extract type	Average Phenolic content (mgGAE/g Extract ± SD)
n-Hexane	0,57 ± 0,02
Ethyl Acetate	2,95 ± 0,07
Ethanol 70%	16,71 ± 0,43

Table 5. Results of Total Flavonoid Levels

Extract type	Average levels of Flavonoids (mgQE/g Extract ± SD)
n-Hexane	55,57 ± 0,11
Ethyl Acetate	88,79 ± 0,73
Ethanol 70%	6,23 ± 0,05

Determination of total flavonoid levels uses quercetin standards. Quercetin is a flavonoid class of the flavonol group [23]. Meanwhile, Quantitative Analysis is conducted by applying the UV-Vis spectrophotometric method (Hanani, 2015). The maximum wavelength resulted from this study was 434 nm. This was due to the reaction of the AlCl3 complex which caused the shift of the wave to become visible, which was indicated by the solution becoming more yellow, and the addition of sodium acetate to maintain the visible wavelength [24]. $AlCl_3$ reagents with flavonoids form complexes between neighboring hydroxyl groups and ketones or with neighboring hydroxyl groups. $AlCl_3$ will react with the ketone group at C4 and the OH group at C3 or C5 in flavone or flavonoid compounds to form a yellow stable complex compound.

The results of the absorbance measurement of the quercetin standard solution to obtain a linear calibration curve obtained results such as (Table 6). These results were entered into the regression equation which then produced linear y = 0.0024x+0.1897,r = 0.9931. The flavonoid content was expressed in mgQE/g Extract.

After reading the levels of flavonoids (Table 7) of the graded extract of katuk leaves, the results obtained were *n*-hexane 55.57 mgQE/g, ethyl acetate 88.79 mgQE/g, and 70% ethanol 6.23 mgQE/g. In this study, ethyl acetate had the largest concentration, this is because the content of flavonoid compounds contained in katuk leaves has a low polarity level, namely in the form of aglycones or commonly called aglycone flavonoids. Aglycone flavonoids have less polar properties and tend to be more soluble in chloroform and ether. Flavonoids generally bind to sugars to form glycosides which cause these compounds to be easily soluble in polar solvents, such as methanol, butanol, and ethyl acetate.

In this study, the free radical compound DPPH (2,2-*diphenyl*-1-*picrylhydrazyl*) was used. The results obtained on the measurement of the maximum wavelength is 515.5 nm, the wavelength can be used because the wavelength of the maximum absorbance for measurements with the DPPH method is 515-520 nm [25].

Quercetin was chosen as the standard for comparison because quercetin is a natural secondary antioxidant that has been shown to have free radical scavenging activity. Quercetin, which is a flavonoid group, has several biological activities. These activities can be attributed to the antioxidant properties of quercetin, including its ability to scavenge free radicals [23].

The results of antioxidant activity (Table 8) show that 70% ethanol extract has a lower IC_{50} value than ethyl acetate and *n*-hexane, the smaller the % inhibition obtained, the stronger the antioxidant activity produced [7]. The compounds contained in the ethanol extract are an accumulation of polar, semi-polar, and non-polar compounds. When the extract is macerated in stages, the synergistic function

between the compounds will reduce because the components contained in the extract have been separated. The non-polar chemical components were extracted in the *n*-hexane solvent, the semi-polar chemical components were extracted in ethyl acetate, and chemical components that are polar were extracted in 70% ethanol solvent. This causes the antioxidant activity of 70% ethanol extract to be the strongest and at the same time has the least % inhibition, compared to others.

Table 6. Antioxidant Activity Test Results and SPF

Sample Type	Average antioxidant activity (IC_{50} ± SD)	SPF
Quercetin	8,83	-
n-Hexane	88,43 ± 1,20	2,13
Ethyl Acetate	77,65 ± 1,78	5,23
Ethanol 70%	70,33 ± 1,64	2,13

Ethyl acetate extract gave the highest SPF results compared to ethanol and *n*-hexane extracts. The three extracts belonged to the minimum SPF capability (range 2 to 4). Phytochemical compounds in the extract that may play a role in the potential for absorption of ultraviolet light are flavonoids and phenolic compounds [26]. Several groups of active compounds derived from natural ingredients such as flavonoids, tannins, anthraquinones, cinnamates, and glycosides are reported to have the ability to protect against UV rays [27].

4. Conclusions

Based on the results obtained in this study, the determination of total phenolic content had the highest concentration in 70% ethanol extract, which was then followed by ethyl acetate extract and *n*-hexane extract. In the determination of total flavonoid content, the highest yield was in the ethyl acetate extract, then *n*-hexane content, while the 70% ethanol had the lowest content. The results of the antioxidant activity test used the DPPH method. IC_{50} of *n*-hexane extract had the highest concentration in this activity test, followed by ethyl acetate and 70% ethanol extract. The smaller the IC_{50} value, the higher the antioxidant activity produced. Multilevel extraction greatly affects the levels of each extract used. Besides, it will also look for different compounds in the solvent used.

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Untuk Melaksanakan Penelitian dan Publikasi sebagai berikut:

NO	JUDUL PENELITIAN DAN PUBLIKASI
1.	**Identifikasi Simultan Sildenafil Sitrat dan Tadalafil pada Kopi Herbal menggunakan Kromatografi Lapis Tipis – Densitometri**

Demikian surat tugas ini diberikan kepada yang bersangkutan untuk dilaksanakan dengan penuh amanah dan tanggung jawab

Jakarta, 09 September 2022
Dekan,

Dr. apt. Hadi Sunaryo, M.Si., Apt.

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Identifikasi Simultan Sildenafil Sitrat dan Tadalafil pada Kopi Herbal menggunakan Kromatografi Lapis Tipis – Densitometri

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Abstract: In Indonesia, people frequently turn to herbal remedies as an alternative to conventional medicine. For more convenient use, herbs are currently packed as coffee or other powdered drinks. Because the transactions were more frequent and extensive, sildenafil and tadalafil were found in herbal coffee sold in internet retailers in this investigation. Thin-layer chromatography densitometry tests were conducted on samples of herbal coffee and the standard using ethyl acetate-methanol-ammonia as the mobile phase. The method's selectivity demonstrated that sildenafil and tadalafil in herbal beverages may be distinguished using this technique. Four samples from the ten herbal coffee samples tested positive for sildenafil, and four samples tested positive for tadalafil, according to the test results.

Keywords: Coffee, Chromatography, Herbal, Sildenafil, Tadalafil.

Abstrak: Di Indonesia, masyarakat sering beralih ke pengobatan herbal sebagai alternatif pengobatan konvensional. Agar lebih nyaman digunakan, jamu saat ini dikemas dalam bentuk kopi atau minuman bubuk lainnya. Karena transaksi lebih sering dan luas, sildenafil dan tadalafil ditemukan dalam kopi herbal yang dijual di pengecer internet dalam penyelidikan ini. Uji densitometri kromatografi lapis tipis dilakukan pada sampel kopi herbal dan standar menggunakan etil asetat-metanol-amonia sebagai fase gerak. Selektivitas metode menunjukkan bahwa sildenafil dan tadalafil dalam minuman herbal dapat dibedakan dengan menggunakan teknik ini. Empat sampel dari sepuluh sampel kopi herbal dinyatakan positif sildenafil, dan empat sampel dinyatakan positif tadalafil, menurut hasil tes.

Kata Kunci: Herbal, Kromatografi, kopi, Sildenafil, Tadalafil.

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1. PENDAHULUAN

Minuman herbal merupakan minuman tradisional yang mempunyai beberapa manfaat jika dikonsumsi. Minuman herbal terdiri dari beberapa bahan tumbuhan yang bermanfaat bagi kesehatan. Di Indonesia, jamu umumnya digunakan sebagai alternatif penggunaan obat modern pada masyarakat ekonomi rendah, menengah, dan atas hingga mencapai 58 persen (Andriati & Wahjudi, 2016). Saat ini herbal juga dikemas dalam bentuk kopi atau minuman serbuk lainnya sehingga lebih memberikan rasa nyaman dalam konsumsinya.

Menurut Peraturan Menteri Kesehatan Republik Indonesia Nomor 007 Tahun 2012 tentang registrasi obat tradisional yang menyatakan bahwa Obat tradisional dilarang mengandung bahan kimia obat yang merupakan hasil isolasi atau sintetik berkhasiat obat (Kementrian Kesehatan RI, 2012). Bahan kimia obat merupakan zat-zat kimia yang digunakan sebagai bahan utama obat kimiawi yang biasanya ditambahkan dalam sediaan obat tradisional/jamu untuk memperkuat indikasi dari obat tradisional tersebut. Jamu dengan kandungan bahan kimia obat menyebabkan citra jamu sebagai budaya Indonesia, menjadi buruk. Bahan kimia obat yang sering ditambahkan ke dalam jamu antara lain: Fenilbutazon, antalgin, diklofenak sodium, piroksikam, parasetamol, prednison, deksametason, sibutramin hidroklorida, sildenafil sitrat, glibenklamid, dan teofilin (BPOM Padang, 2021).

Pada tahun 2020 beberapa produk ilegal dimusnahkan salah satunya obat tradisional ilegal sebanyak 221 produk (46,13%). Dari produk-produk tersebut terdapat jamu yang mengandung bahan kimia obat yaitu Sildenafil Sitrat (BPOM RI, 2020). Obat herbal perkasa pria, atau obat kuat seperti yang lebih dikenal di masyarakat, telah banyak digunakan di masyarakat, baik dengan maupun tanpa nomor ijin edar BPOM. Badan Pengawas Obat dan Makanan (BPOM) sering melihat penyalahgunaan jamu kuat ini dan yang paling banyak adalah penambahan bahan kimia obat 9BKO) yaitu Sildenafil Sitrat (BPOM RI, 2016). Sildenafil sitrat termasuk golongan obat keras yang hanya dapat diperoleh dengan resep dokter. Sildenafil merupakan salah satu bahan aktif yang digunakan dalam pengobatan disfungsi ereksi atau lebih dikenal dengan impotensi inhibitor dalam kelompok penghambat phosphodiesterase. Selain sildenafil, juga digunakan pada hipertensi pulmonal (PAH) (Mehta, 2023)

Beberapa peneliti yang telah melakukan penelitian mengenai kandungan sildenafil sitrat pada jamu kuat. Salah satunya pada penelitian yang dilakukan (Waris et al., 2013) yang mendapatkan hasil dari 4 sampel jamu kuat yang dianalisis terdapat 1 sampel yang positif mengandung sildenafil sitrat, penelitian ini menggunakan metode TLC-densitometri. Penelitian yang telah dilakukan oleh (Sarigih et al., 2010) mendapatkan hasil positif mengandung BKO sildenafil sitrat pada jamu perkasa merek A menggunakan metode Kromatografi Cair Kinerja Tinggi.

Pada penelitian dilakukan analisis kandungan Sildenafil Sitrat dan Tadalafil pada kopi herbal menggunakan alat KLT-Densitometri. Kromatografi lapis tipis merupakan Teknik kromatografi yang berguna untuk memisahkan senyawa organik. Karena kemudahan dan kecepatan penggunaan KLT, metode ini banyak digunakan untuk memantau perkembangan reaksi sintesa organik dan untuk memeriksa kemurnian produk. Teknik penggunaan KLT memiliki banyak keuntungan karena KLT merupakan teknik serbaguna yang dapat diterapkan pada hampir semua senyawa. Karena adsorben yang baik dan pelarut yang bersih, pemisahan dapat dicapai dengan biaya rendah. Pemisahan dapat dicapai dalam waktu singkat, menjadikan KLT sebagai teknik yang dijamin berhasil untuk memisahkan campuran yang tidak diketahui (Rosamah, 2019). KLT juga memiliki tingkat ketelitian yang tinggi sehingga menghasilkan pemisahan yang lebih sempurna dan tingkat kepekaan yang tinggi (Asmawati et al., 2019).

2. MATERIAL AND METODA

2.1 Alat dan Bahan Penelitian

Alat yang digunakan dalam penelitian ini adalah neraca analitik, peralatan gelas, silika gel 60 GF 254, chamber, oven, lemari asam, hair dryer, microliter syringe, mikropipet, pensil, penggaris dan UV-Visible 254 nm dan Densitometer. Bahan yang digunakan dalam penelitian ini adalah sampel kopi herbal yang diperoleh dari *online shop* Shopee, metanol 99,8% (Merck), kloroform (Merck), etil asetat(Merck), propanol (Merck), standar sildenafil sitrat (BPOM), standar Tadalafil (BPOM).

2.2 Metode Penelitian

Metode penelitian terdiri dari pengumpulan sampel kopi herbal, pembuatan sampel simulasi, optimasi fase gerak, uji selektivitas, LOD, dan uji sampel kopi herbal.

a. Pengumpulan sampel kopi herbal

Pemilihan dan pembelian sampel ini dilakukan secara

purposive sampling, yaitu adalah salah satu cara menentukan sampel dengan pertimbangan tertentu (Sugiyono, 2018). Pemilihan sampel kopi dilakukan pembelian di *online shop* Shopee dengan kriteria sebagai berikut:

Kriteria inklusi sampel jamu kuat pria

a. Kopi yang memiliki atau tidak memiliki nomor izin edar BPOM atau tidak terdaftar di BPOM, tetapi mencantumkan nomor izin edar fiktif pada kemasan.
b. Kopi mempunyai kandungan herbal.
c. Kopi menyatakan narasi "Kuat", "Tahan Lama", dan/atau "Perkasa".
d. Produk yang terjual di toko sudah lebih dari 1000 produk.

b. Pembuatan sampel kopi herbal simulasi

Kopi herbal dibuat dengan komposisi :

1. Pasak bumi (*Eurycoma longifolia*) 1,25 gr
2. Ginger powder (*Zingiberis rhizoma*) 1,25 gr
3. Purwoceng (*Pimpinella pruatjan*) 1 gr
4. Ginseng (*Panax*) 0,25 gr
5. Tribulus (*Tribulus terrestris*) 0,25 gr
6. Kopi dan Gula ad 25 gr

c. Pembuatan Larutan Baku

Baku sildenafil dan baku tadalafil masing-masing dilarutkan dalam metanol dengan konsentrasi 1000 ppm.

d. Optimasi fase gerak

Pengujian berbagai macam variasi fase gerak yang terdiri dari methanol, etanol, etil asetat, asetonitril, amonia, diklorometana pada plat silika gel 60 GF254 yang berisi baku sildenafil, baku tadalafil, kopi herbal simulasi dan *spiked* kopi herbal.

e. Uji Selektifitas

Pengujian menggunakan fase gerak yang paling optimum pada plat silika gel 60 GF254 yang berisi baku sildenafil, baku tadalafil, baku campuran, kopi herbal simulasi dan *spiked* kopi herbal.

f. Limit Deteksi

Pengujian menggunakan fase gerak yang paling optimum pada plat KLT yang berisi baku sildenafil konsentrasi 20, 30, 40, 50 dan 60 ppm sedangkan untuk baku tadalafil 5, 10, 15, 20 dan 30 ppm. Hasil AUC puncak dihitung dengan metode standar deviasi residual.

g. Preparasi dan pengujian sampel

Sampel serbuk kopi herbal sebanyak 400 mg dimaserasi 1 – 2 hari dengan metanol 5 ml. Ekstrak jamu disaring dan ditampung (Waris et al., 2013). Plat silika gel 60 GF 254 disiapkan kemudian menotolkan baku pembanding sildenafil sitrat, baku pembanding tadalafil, baku campuran dan 10 ekstrak sampel kopi herbal ditotolkan masing-masing 5 µL dengan jarak antar totolan 1 cm. Selanjutnya dilakukan elusi pada fase gerak optimum, diamati dengan bantuan sinar *Ultra Violet* (UV) 254 nm.Noda yang terbentuk pada senyawa pembanding dengan ekstrak dan standar dibandingkan jarak rambatnya. Selanjutnya diukur menggunakan KLT-Densitometri pada panjang gelombang maksimum 292 nm, dan dilakukan analisis terhadap hasil scan (Waris et al., 2013).

3. HASIL DAN DISKUSI

Sampel kopi herbal yang didapatkan sebanyak 10 kopi herbal. Penelusuran Nomor Ijin Edar (NIE) melalui website BPOM ditemukan bahwa 5 sampel

mempunyai NIE yang sesuai label kemasan, 1 sampel NIE fiktif dan 4 sampel tidak mempunyai NIE.

Kopi herbal simulasi dibuat dengan cara mencampurkan beberapa herbal yang tercantum dalam komposisi kopi herbal sampel hasil pencarian di *online shop* Shopee. Komposisi herbal antara lain pasak bumi, jahe, purwoceng, ginseng dan tribulus.

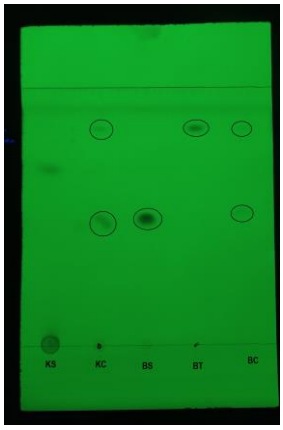
Gambar 1: Kopi Herbal Simulasi

Optimasi fase gerak menghasilkan fase gerak etil asetat:methanol:amonia (45:5:1) yang paling optimum. Fase gerak tersebut dapat memberikan noda baku sildenafil, baku tadalafil, dan *spiked* kopi herbal simulasi yang terpisah dengan baik.

Tabel 1: Hasil Optimasi Fase Gerak

Fase Gerak	**Baku Sildenafil**	**Baku Tadalafil**	**Kopi Simulasi**	***Spiked* Kopi Simulasi**
Metanol : Kloroform 4:1	Tampak	-	-	-
Kloroform:Metanol:Amonia (70:3;1,5)	Tampak	Tampak	-	-
Etil asetat: Asetonitril: Ammonia (45:5:1)	Tampak	Tampak		Tampak (hanya sildenafil)
Etil asetat: Metanol: Ammonia (45:5:1)	Tampak	Tampak		Tampak
Kloroform: Metanol: Ammonia (45:5:1)	Tampak	Tampak		
Diklormetan: Metanol: Ammonia (45:5:1)	Tampak	Tampak		Tampak (sildenafil *tailing*)

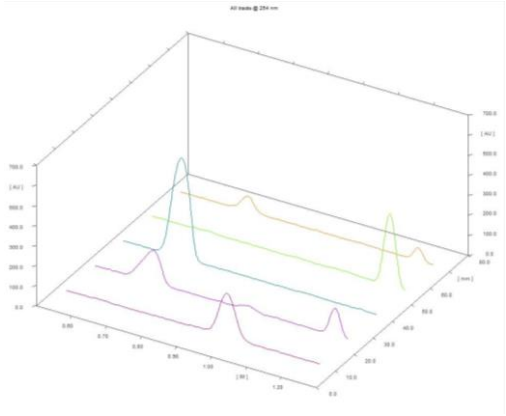
Hasil selektivitas menunjukkan pemisahan yang baik untuk spiked kopi herbal simulasi. Tadalafil dan sildenafil dalam spiked kopi herbal memberikan Rf dan panjang gelombang yang sama dengan baku sildenafil dan baku tadalafil. Pada sampel kopi herbal simulasi menunjukkan noda pada Rf 0,98 yang diasumsikan sebagai kafein dari komposisi kopi dalam sampel tersebut karena sesuai dengan Panjang gelombang ultraviolet kafein yaitu 273 nm (Dibbern et al., 2002)



Gambar 2: Hasil plat silica gel 60 GF254 uji selektivitas menggunakan fase gerak etil asetat:methanol:ammonia (45:5:1) (KS:kopi simulasi, KC:Spiked kopi simulasi, BS:Baku Sildenafil, BT:Baku Tadalafil, BC:Baku Campuran)

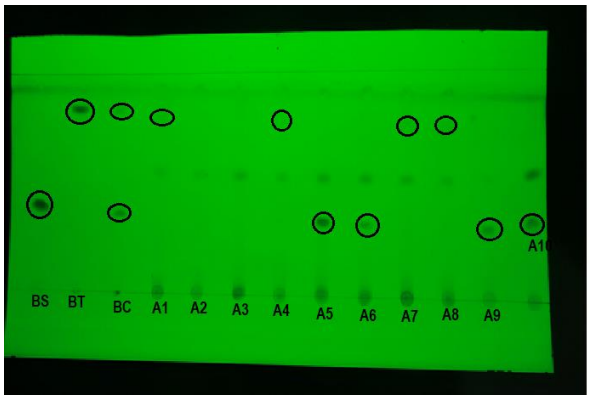
Tabel 2: Hasil Uji Selektifitas

Sampel	**Senyawa**	**Rf**	**λ maks**
Kopi Simulasi	Kafein Kopi	0.98	275
Spiked Kopi Simulasi	Sildenafil	0.69	304
Spiked Kopi Simulasi	Kafein Kopi	0.97	275
Spiked Kopi Simulasi	Tadalafil	1.21	200
Baku Sildenafil	Sildenafil	0.69	304
Baku Tadalafil	Tadalafil	1.21	200
Baku Campuran	Sildenafil	0.72	305
Baku Campuran	Tadalafil	1.21	200



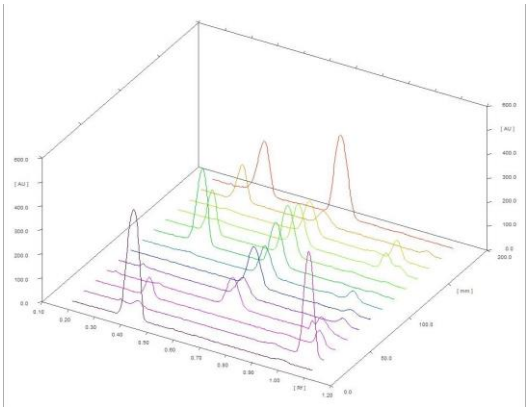
Gambar 3: Plot spektrum hasil selektivitas

Limit deteksi dilakukan menggunakan metode eksperimen dengan jalan membuat rentang kadar baku sildenafil atau tadalafil sebanyak 5 konsentrasi dan setiap konsentrasi diukur luas areanya. Perhitungan limit deteksi menggunakan rumus 3.3*SD/S (European Medicines Agency, 1995). Limit Deteksi untuk sildenafil adalah 8,8 ppm. Limit deteksi untuk tadalafil adalah 5,0 ppm.



Gambar 4: Hasil uji plat silica gel 60 GF254 sampel kopi herbal menggunakan fase gerak etil asetat:methanol: ammonia (45:5:1) (BS:Baku Sildenafil, BT:Baku Tadalafil, BC:Baku Campuran, A1-A10:Sampel Kopi Herbal)

Hasil pengujian sampel adalah semua sampel kopi memberikan noda pada Rf kafein yang merupakan bahan aktif dari serbuk kopi. Dari 10 sampel kopi herbal tersebut menunjukkan bahwa 4 sampel positif tadalafil pada sampel A1, A4, A7 dan A8 yang memiliki noda pada Rf yang sama dengan baku tadalafil dan panjang gelombang 287 nm. Sampel positif sildenafil sebanyak 4 sampel yaitu A5, A6, A9 dan A10 yang memiliki noda pada Rf yang sama dengan baku sildenafil panjang gelombang 305 nm. Peneliti juga telah melaporkan sampel kopi yang terdeteksi positif baik sildenafil maupun tadalafil ke Badan POM melalui mekanisme Unit Layanan Pengaduan Konsumen (ULPK).



Gambar 5: Hasil uji sampel kopi herbal pada Kromatografi Lapis Tipis-Densitometri

4. KESIMPULAN

Kromatografi Lapis Tipis – Densitometri dengan pelarut etil asetat – metanol – amonia mampu mendeteksi sildenafil dan tadalafil pada sampel kopi herbal. Dari 10 sampel yang dianalisis, 4 sampel terdeteksi mengandung sildenafil dan 4 sampel terdeteksi mengandung tadalafil.

5. UCAPAN TERIMAKASIH

Terima kasih kepada Lemlitbang UHAMKA atas pendanaan riset ini.

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