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Stability Testing and Determination of *Sun Protection Factor* (SPF) Value in Gel Formulation Combining *Moringa oleifera* L. Leaf Extract with *Citrus aurantifolia* Peel

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Abstract

The Sun Protection Factor (SPF) is an indicator that elucidates the effectiveness of a substance as a UV protector. One of the factors that influences the SPF value is the stability of the formulation. A plant with the potential to serve as a sunscreen, exhibiting both a high SPF value and gel stability, is a combination of Moringa leaves (Moringa oleifera L.*) and lime peel (Citrus aurantifolia). The objective of this study is to assess the SPF value of gel preparations containing a combination of Moringa leaves and lime peel, as well as the stability of these gel preparations before and after storage, in accordance with the requirements of SNI 16-4399-1996. Three combinations, namely 1:1 (F 1), 1:2 (F II), and 2:1 (F III), were evaluated. The method employed in this study is maceration using a solvent of 96% ethanol. The results of the physical evaluation, including spreadability, pH, and adhesion, before and after stability testing for formulations F I, F II, and F III, demonstrated no significant differences with a P-value < 0.05. This indicates that the three formulations exhibited comparable results. The highest SPF test results among the three formulations, both before and after stability testing, were observed in formula III, which exhibited an SPF value of 23.86 ± 0.19. This value falls within the ultra category, and the difference was statistically significant with a P-value < 0.05.*

I. Introduction

The Sun Protection Factor (SPF) is an indicator that explains the effectiveness of a substance that acts as a UV protector. The higher the SPF value of an active substance, the more effective it is in protecting the skin from the harmful effects of UV rays (Salsabila et al., 2021). The effectiveness of preparations is classified based on the SPF value, namely non-sunscreen (SPF < 2), minimum potency (SPF 2-11), moderate potency (SPF 12-30), and high protection (SPF ≥ 30) (Cahyani & Erwiyani, 2022).

Moringa leaves (*Moringa oleifera* L.) are a type of tropical plant that is easily recognizable due to its small leaf size. Moringa leaves also grow very easily in soil that can be considered less fertile (Slamet *et al*., 2020). The study by Sari (2018) found that the 50% ethanol extract of moringa leaves has a Sun Protection Factor (SPF) activity of 24.75, which falls within the ultra category. In addition to moringa leaves, lime peel also exhibits ultra SPF activity.

Lime (*Citrus aurantifolia*) is a type of plant belonging to the citrus family, which is widely distributed in Asia and Central America. It is also known as "jeruk pecel" (Jazilatun, 2019). Research conducted by Afiddah et al (2016) found that the SPF content in lime peel extract yielded a high SPF value of 40.15 at a concentration of 300 ppm, which falls into the ultra protection category.

The combination of moringa leaves (*Moringa oleifera* L.) and lime peel (*Citrus aurantifolia*) is still rarely used by the community for formulation purposes. When the extracts or fractions of moringa leaves and lime peel are combined, they can result in improved SPF values. The extraction method used in this study was maceration on moringa leaves (*Moringa oleifera* L.) in combination with lime peel (*Citrus aurantifolia*) using 96% ethanol solvent with ratios of 1:1, 1:2, and 2:1 (Rudiana *et al*., 2020).

Based on the information above, a study will be conducted to assess the stability and determine the Sun Protection Factor (SPF) value of a gel formulation combining moringa leaf extract (*Moringa oleifera* L.) with lime peel (*Citrus aurantifolia*). The aim is to determine the optimal SPF value in the gel formulation.

II. Research Method

II.1 Equipment/Instruments

The equipment/instruments used in this research are an analytical balance, 100 mL Pyrex®

glass beaker, 500 mL Pyrex® *glass beaker*, 5 mL Pyrex® measuring *cylinder*, 10 mL Pyrex® measuring *cylinder*, 50 mL Pyrex® measuring *cylinder*, 100 mL Pyrex® measuring *cylinder*, pH meter, *Rheosys merlin viscometer, spreading apparatus, glass slides, glass coverslips, spatula, Petri dish, ruler,* Pyrex® test tube, test tube rack, *rotary evaporator, water bath, mortar and pestle, stir rod, blender, Thermo Scientific* UV-Vis *spectrophotometer, cuvette, and stirring rod* (Ulfa & Ismawati, 2016).

II.2 Material

The materials used in this research are moringa leaf extract (*Moringa oleifera L.*), lime peel extract (*Citrus aurantifolia*), *Hydroxypropyl methylcellulose* (HPMC), *Triethanolamine* (TEA), *Propyl paraben, Methyl paraben, Propylene glycol, Glycerin, Distilled water, and 96% ethanol* (Ulfa & Ismawati, 2016).

II.3 Collection and Processing of Simplified Herbs

The collection and processing of 8 kg of moringa leaves were obtained from the surroundings of Universitas Malahayati, Bandar Lampung, and 5 kg of lime peel were obtained from Menggala Subdistrict, Tulang Bawang. The moringa leaves and lime peel were cleaned and washed with running water. They were then sliced and air-dried for 30 days. After drying, they were ground using a blender and sieved. The obtained powdered simplisia was placed in each jar, with a quantity of 600 g for maceration (Suryadi *et al*., 2021).

II.4 The Production of Moringa Leaf Extract and Lime Peel Extract

The Moringa leaves and lime peel, which have been processed into powdered simplisia, are then extracted through maceration using 96% ethanol as a solvent. Each batch of powdered simplisia, weighing 600 grams, is soaked in 7 liters of 96% ethanol solvent individually for a duration of 3 x 24 hours. Subsequently, the mixture is filtered using filter paper to obtain a macerate. The macerate is subjected to evaporation using a rotary evaporator at a temperature of 50ºC until a concentrated extract is obtained (Lestari & Amalia, 2018).

II.5 The Preparation of Gel Formulation

The preparation of the gel begins by preparing the weighed ingredients according to the formula. Water is heated and then added to a bowl containing a mortar. The gelling agent (HPMC) is added to the mortar, followed by the addition of preheated distilled water. The mixture is allowed to swell for 15 minutes and then ground until homogeneous. Methyl paraben and propyl paraben are dissolved in glycerin and added to the gel mass, followed by further grinding until homogeneous. Propylene glycol is gradually added to the gel mass while grinding until homogeneous. Then, triethanolamine is added to the gel mass and ground until homogeneous, resulting in the formation of the gel mass. The combination of Moringa leaf extract and lime peel extract is added to the gel mass in three different ratios 1:1, 1:2, and 2:1. The formula used is presented in Table 1.

II.6 SPF Testing (Sun Protection Factor)

SPF value testing was conducted in vitro using a UV-Vis spectroscopy instrument. Each sample was weighed at 1 gram and diluted with ethanol in a 25 mL volumetric flask, then filtered using filter paper. A 5 mL aliquot was pipetted and transferred into a 10 mL volumetric flask, then diluted with ethanol. The obtained solution was measured using UV-Vis spectroscopy at a wavelength range of 290-320 nm, using ethanol as the blank. The absorbance values were recorded at every 5 nm interval (Ismail *et al*., 2014). The obtained absorbance results were calculated using the Mansur equation to determine the SPF value (Mansur *et al*., 1986). The testing of the Sun Protection Factor (SPF) value was conducted using a UV-Vis spectroscopy instrument, and the data was processed using the formula equation (1) Mansur for SPF determination.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda)\, I(\lambda)\, Abs(\lambda) \qquad (1)$$

Description CF: Correction (10); EE: Erythemal effect spectrum*;* I: Intensity spectrum*;* Abs: Absorbance.

The standard value of EE x I is used to calculate the SPF value. Absorbance is measured at wavelengths of 290, 295, 300, 305, 310, and 320 nm. The obtained absorbance values are multiplied by the corresponding EE x I values for each wavelength. The multiplied results of absorbance and EE x I are summed. The sum is then multiplied by a correction factor to obtain the SPF value of the formulation.

II.7 Evaluation of the Gel Formulation

II.7.1 Organoleptic Test

Organoleptic observations include the examination of the shape, color, and odor of the gel formulation, which are visually assessed *(Sinala et al*., 2019).

II.7.2 Spreadability Test

The gel formulation is placed on glass A, which has dimensions of 20 x 20 cm, with a weight of 1 gram. Then, glass A is covered with glass B, and a weight of 100 grams is placed on top of it. After 1 minute, the diameter is measured. The requirement for good spreadability on the skin is 5-7 cm (SNI, 1996).

II.7.3 pH Test

The prepared gel formulation is taken at 1 gram and dissolved in distilled water (Istiana et al., 2021). The pH testing is performed by A universal pH strip is used, which is inserted into the sample dissolved in distilled water. The requirement for good skin pH is between 4.5 and 8 (SNI, 1996).

II.7.4 Homogeneity Test

The gel formulation is weighed at 50 mg and then placed in another *glass object*. Strong pressure is applied to both *glass objects*, and they are observed (Mardhiyani, 2022). The formulation is considered homogeneous if its color is consistent, and there are no particles or coarse materials present (Saputra *et al*., 2019).

II.7.5 Adhesion Test

The gel formulation is weighed at 0.5 grams and placed on the glass object for the adhesion test. A 500-gram weight is applied to the glass object, left undisturbed for 1 minute, and then the weight is removed. The time is recorded until both glass objects detach. Good adhesion for the skin is defined as ≥ 1 second (SNI, 1996).

II.7.6 Viscosity Test

The viscosity test is conducted to determine the level of sample thickness, and it utilizes the *Rheosys Merlin viscometer*. The gel formulation is weighed at 1.5 grams and placed in the spindle. The viscometer is turned on, and the cPs value displayed on the monitor is recorded. The requirement for good skin viscosity is between 2,000 and 50,000 cps (SNI, 1996).

III. Results and Discussion

Below, the results of the conducted research can be observed from several tests, including extraction (Table 2), organoleptic test (Table 3), homogeneity test (Table 4), spreadability, pH, adhesion, and viscosity tests (Table 5), as well as SPF testing (Table 6).

Table 1. Formulation of gel preparation with moringa leaf and lime peel extract combination

Materials	Consentration		
	F1	F2	F3
Combination of Moringa (*Moringa oleifera* L.) leaf extract with Lime (*Citrus aurantifolia*) peel extract	1 : 1	1 : 2	2 : 1
HPMC (Hydroxypropyl methylcellulose)	2	2	2
Glycerin	5	5	5
Propylene glycol	2,5	2,5	2,5
Methyl paraben	0,2	0,2	0,2
Propyl paraben	0,05	0,05	0,05
TEA (Triethanolamine)	2	2	2
Distilled water	100 mL	100 mL	100 mL

Table 2. Results of extraction of *Moringa oleifera* L. leaves and *Citrus aurantifolia* lime peel

Material	Sample Weight (grams)	Concentration of Extract (grams)	Yield (%)
Moringa oleifera Leaf Extract	600	82,07	13,67
Citrus aurantifolia Lime Peel Extract	600	66,44	11,07

Moringa oleifera Leaf powder and *Citrus aurantifolia* Lime Peel powder were each weighed at 600 grams. They were then macerated using 7 liters of 96% ethanol solution for 3 days. The maceration mixture was evaporated using a rotary evaporator at a temperature of 40°C and concentrated in a water bath. As a result, 82.07 grams of *Moringa oleifera* Leaf extract with a yield of 13.67% and 66.44 grams of *Citrus aurantifolia* Lime Peel extract with a yield of 11.07% were obtained. The extraction process conducted for 3 x 24 hours aims to maximize the extraction of chemical compounds present in the samples. Maceration was performed in a dark place to reduce the risk of reactions between the materials in the bottle and sunlight.

Table 3. Results of organoleptic observation of the gel formulation

Organoleptic Evaluation of the Gel Formulation					
Before Stability Test			**After Stability Test**		
Sample Code	**Colour**	**%**	**Sample Code**	**Colour**	**%**
F I	Brownish Yellow	60%	F I	Brownish Yellow	65%
F II	Yellow	80%	F II	Yellow	90%
F III	Pale Brown	90%	F III	Pale Brown	90%
Sample Code	**Smell**	**%**	**Sample Code**	**Smell**	**%**
F I	Distinctive aroma of Moringa leaves and lime peel	55%	F I	Distinctive aroma of Moringa leaves and lime peel	65%

F II	Distinctive aroma of Moringa leaves and lime peel	65%	F II	Distinctive aroma of Moringa leaves and lime peel	75%
F III	Distinctive aroma of Moringa leaves and lime peel	85%	F III	Distinctive aroma of Moringa leaves and lime peel	95%
Sample Code	**Shape**	**%**	**Sample Code**	**Shape**	**%**
F I	Gel	65%	F I	Gel	70%
F II	Gel	75%	F II	Gel	80%
F III	Gel	90%	F III	Gel	95%

Organoleptic testing involves the visual examination of the form, color, and odor of the gel formulation (Sinala *et al*., 2019), Before the stability test, the researchers provided a questionnaire to 20 respondents who would observe the gel formulation. The questionnaire consisted of 3 questions, each with 4 columns for selection.

Before and after stability testing, the organoleptic evaluation revealed that the most preferred color was found in Formula III, described as pale brown. The most preferred scent was also found in Formula III, described as having the distinctive aroma of Moringa leaves and lime peel. The most preferred form was the gel form of Formula III. Organoleptic testing aims to obtain a gel formulation with an appealing color, a scent accepted by users, and a comfortable form for application on the skin.

Organoleptic observations on the combination of moringa leaf extract and lime peel were conducted before stability testing and after stability testing, using 20 individuals as respondents to obtain direct information through a questionnaire method. The questions asked included three physical parameter questions, namely color, smell, and shape, each with four options listed in a table.

The results of the Organoleptic Testing conducted by providing a questionnaire to 20 respondents showed that the majority of them chose the gel formulation I with a brownish yellow color, formulation II with a yellow color, and formulation III with a brownish yellow color. For the smell, the majority of respondents chose the characteristic scent of moringa leaf extract and lime peel for all three formulas. Additionally, the majority of respondents chose the gel formulation for the shape in all three formulas (I, II, and III).

Table 4. Homogeneity test of gel formulations

Replication	**Homogeneity Test**		
	F I	**F II**	**F III**
1	+	+	+
2	+	+	+
3	+	+	+

Homogeneity test is a test conducted to determine the presence or absence of coarse particles in a formulation. The homogeneity test of gel formulations is performed to assess the homogeneity and mixing process of each component or ingredient in gel production (Jufri *et al*., 2006).

The homogeneity test is conducted by placing the gel formulation inside an object glass and covering it with another object glass. All three gel formulations that were prepared exhibited homogenous mass, as the gel production process involved continuous and constant stirring, resulting in a homogenous gel mass (Saputra *et al*., 2019).

Table 5. Spreadability test, pH, adhesion, and viscosity test of gel formulations

Formula	**Parameter**	**Observation of Test Results**		**P-*Value***	**According to the Indonesian National Standard (SNI 16-4399-1996)**
		Before Stability Testing	**After Stability Testing (12 Days)**		
F I	Spreadability	5.1 ± 0.30	6.1 ± 0.2	0.45	
	pH	7.8 ± 0.35	7.6 ± 0.46	0.38	**Spreadability: 5-7 cm**
	Adhesion	1.52 ± 0.39	2.30 ± 0.07	0.40	
	Viscosity	9.472 cps ± 95.25	9.587cps ± 60.14	0.04	
F II	Spreadability	5.4 ± 0.25	5.1 ± 0.7	0.61	**pH: 4,5-8**
	pH	7.8 ± 0.70	7.5 ± 0.15	0.65	
	Adhesion	1.66 ± 0.37	2.29 ± 0.09	0.05	
	Viscosity	2.980 cps ± 30.79	3.488 cps ± 48.83	0.10	**Adhesion: ≥ 1 second**
F III	Spreadability	5.0 ± 0.30	5.6 ± 0.7	0.90	
	pH	7.9 ± 0.95	7.4 ± 0.15	0.98	

	Adhesion	1.52 ± 0.32	2.25 ± 0.10	0.54	**Viscosity:**
	Viscosity	2.316 cps ± 31.26	3.741 cps ± 46.36	0.04	**2.000-50.000 cp**s

The spreadability test is the ability to measure the speed of spread of a gel formulation when applied to the skin (Sinala *et al*., 2019). The spreadability test is performed using instruments such as Glass A and Glass B, which have dimensions of 20 x 20 cm, and a weighing scale with a weight of 100 grams.

Good spreadability is one of the indicators that a gel formulation is easily applied. If a formulation has high spreadability, it means that it can cover a larger area, resulting in the even distribution of the active ingredients and enhancing its therapeutic effects effectively.

The result of a good spreadability test is one of the indicators that the gel formulation is easy to apply. If a formulation has high spreadability, it means that it can cover a larger area, resulting in the even distribution of the active ingredients and making it more effective in producing therapeutic effects. A gel formulation that is difficult to spread or spreads too much will reduce the level of user comfort and the effectiveness of product application. The spreadability test aims to determine the smoothness of the gel formulation on the skin. The longer the storage time of the gel formulation, the more it tends to become thinner or less viscous due to the inability of the base to retain the water penetrated into the formulation.

The results of the Paired Samples T-Test for the test of dispersion indicate that there is no significant difference between formulas I, II, and III before stability and after stability, as the P-Value is greater than 0.05.

The testing of pH value is an acidity measurement performed on a sample to determine the pH of a formulation (Sinala *et al*., 2019). pH value testing is conducted as a physicochemical parameter of a substance that is crucial in gel formulations due to its relevance to the effectiveness and stability of the active ingredient in the gel formulation. pH testing is performed using a pH meter.

The measurement of pH value aims to determine whether the produced gel formulation is compatible with the skin's pH or not, as this is related to the safety and comfort of the formulation during usage. Changes in pH during storage indicate the instability of the formulation, which can result in product deterioration during storage or use. pH changes can be caused by several factors, including light exposure, temperature, and air humidity.

The pH testing results of the formulation during storage indicate its insufficient stability during storage, and this instability can potentially damage the product during storage or usage. Changes in pH can be caused by several factors, including light exposure, temperature, and air humidity.

The results of the Paired Samples T-Test for the pH test indicate that there is no significant difference between formulas I, II, and III before stability and after stability, as the P-Value is greater than 0.05.

Adhesion testing is the ability of a gel to adhere to the skin surface within a specific period of time, enabling optimal functionality in delivering the active ingredient (Istiana *et al*., 2021). The adhesion test is conducted using a glass object with a 500-gram weight.

A good gel formulation ensures effective contact time with the skin to achieve its intended purpose, while also avoiding excessive stickiness for user comfort during application. The longer the time required for the glass objects to detach, the higher the adhesion power, indicating that the formulation adheres to the skin for a longer duration, resulting in prolonged effects of the active ingredient.

The results of the adhesion testing before and after stability indicate that all gel formulation formulations meet the adhesion requirement of ≥ 1 second (SNI, 1996). The adhesion testing experienced some fluctuations due to temperature variations during storage, but still met the standards for good adhesion.

The results of the Paired Samples T-Test for the adhesion test indicate that formulas I and III, both before and after stability, do not have a significant difference as the P-Value is greater than 0.05. However, formula II shows a significant difference before and after stability as the P-Value is less than 0.05.

Viscosity testing is a method employed to determine the level of thickness or viscosity in a gel formulation (Istiana *et al*., 2021). Viscosity testing is performed using the rheosys merlin viscometer. The viscosity of a gel is typically proportional to the quantity and molecular weight of the thickening agents added.

The results of the viscosity testing before and after stability indicate that all gel formulation formulations meet the requirement of good viscosity for skin, ranging from 2,000 to 50,000 cps (SNI, 1996).

Table 6 shows that the viscosity values before stability testing and after stability testing are as follows: for formula I, it increased from 9,472 cps to 9,587 cps; for formula II, it increased from 2,980 cps to 3,488 cps; and for formula III, it increased from 2,316 cps to 3,741 cps.

Formula I, both before and after stability, has the highest value compared to formula II and

III. This can be attributed to certain factors such as a higher concentration of the active ingredient, which can result in hydrogen bonding between the hydroxyl (-OH) groups of the polymer and water molecules. As a result, an increased concentration of the extract leads to more hydroxyl groups, which in turn contributes to the higher viscosity values in the gel formulation (Nanda *et al*., 2013).

The high viscosity value of formula I compared to formula II and III still falls within the acceptable viscosity range according to SNI 16-4399-1996. Furthermore, it remains stable both before and after stability testing.

The viscosity results indicate that the duration of storage does not have an impact on the viscosity of the gel formulation. All three gel formulations experienced an increase in viscosity during storage using the cycling test method, but the obtained values still comply with the requirements for good viscosity. Therefore, the gel formulations can be considered stable throughout the storage period. The high viscosity value of formula I compared to formula II and III still falls within the acceptable range of viscosity according to SNI 16-4399-1996. Furthermore, it remains stable both before and after stability testing.

The results of the Paired Samples T-Test in the viscosity test indicate that there is a significant difference between Formula I and III before and after stability, as evidenced by a P-Value <0.05. However, Formula II before and after stability does not show a significant difference, as the P-Value >0.05.

The statistical test on Formula I fulfills the physical parameters, while in terms of statistics, only the viscosity has a P-Value that is significant <0.05. Formula II fulfills the physical parameters, while in terms of statistics, only the adhesion strength has a P-Value that is significant <0.05. Formula III fulfills the physical parameters, while in terms of statistics, only the viscosity has a *P-Value* that is significant <0.05.

Table 6. SPF testing (Sun Protection Factor)

Formula	SPF Testing			
	Before Stability	**After Stability**	**P-*Value***	(Cahyani & Erwiyani, 2022)
F I	12,20 ± 1,04	22,74 ± 0,04		≤ 2 (not a sunscreen)
F II	15,15 ± 0,09	20,79 ± 0,08	<0,01	2-4 (minimal category)
F III	21,11 ± 0,06	23,86 ± 0,19		4-6 (moderate category)
				6-8 (extra category)
				8-15 (maximum category)
				≥ 15 (ultra category)

The obtained SPF values before and after stability show good SPF values. The calculations for each formula resulted in the following SPF values: Formula I increased from 12.20 (maximum category) to 22.74 (ultra category), Formula II increased from 15.15 (maximum category) to 20.79 (ultra category), and Formula III increased from 21.11 (ultra category) to 23.86 (ultra category).

The measurement results of SPF values were conducted on each formula, and stability testing was performed using the cycling test method for 12 days, with two different temperatures used on each testing day. The SPF data obtained were subsequently analyzed using SPSS through the Paired Samples T-Test, considering a confidence level of 95%. The SPF testing on all formulas before and after stability showed good SPF values, as indicated by a P-Value <0.01. This implies that there is a significant difference between Formula I, II, and III before and after stability.

The stability testing of the gel formulation before and after stability for 12 days showed a decrease and increase in its physical parameters. All formulas that underwent stability testing can still be used as long as they do not exceed the stability period of 12 days. The longer the stability testing is conducted, the product will experience improvement, but it may also result in changes in the quality of the product. During the 12-day period, the gel formulation experienced an increase in its parameters, but it is almost unusable due to the formulation being slightly diluted.

The gel formulation with all three formulas is best stored at room temperature since its physical parameters are not affected. However, if the formulas undergo extended stability testing, they may become unsuitable for application on the skin. The results of the Paired Samples T-Test in the stability testing indicate that, except for Formula I with viscosity parameter, Formula II with adhesion strength parameter, and Formula III with viscosity parameter, there is no significant difference between the formulas before and after stability, as evidenced by a P-Value >0.05. However, the mentioned formulas show a significant difference in their respective parameters, as indicated by a P-Value <0.05.

IV. Conclusions

Based on the conducted research, it can be concluded that the gel formulation containing a combination of moringa leaf extract and lime peel extract fulfills the physical parameters. In terms of statistical analysis, Formula I meets the

requirement for viscosity testing, Formula II meets the requirement for adhesion strength testing, and Formula III meets the requirement for viscosity testing, as indicated by a *P-Value* <0.05, signifying statistical significance. The determination of the best SPF value among the three formulas, both before and after stability, is found in Formula III with an SPF value of 23.86 ± 0.19, which falls under the ultra category. This result is statistically significant, as indicated by a *P-Value* <0.05.

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