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Formulation and SPF Value Evaluation of Sunscreen Spray Gel Containing Lime Peel Extract (*Citrus aurantifolia*)

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Abstract

Background*: High exposure to sunlight has adverse effects on the skin. Lime peel contains more than 60% flavonoids, presenting the potential to function as a sunscreen due to the presence of conjugated aromatic benzene groups, capable of absorbing UV-A or UV-B rays from the sun. To prevent skin damage, lime peel extract is formulated into a spray gel, as it has the ability to dry rapidly, enhancing overall comfort for consumers during application.* ***Objective****: To determine the influence of variation concentration of lime peel extract in the sunscreen spray gel on its physical properties and in vitro SPF value.* ***Methods****: Lime peel crude extract was obtained using 70% ethanol and formulated into a sunscreen spray gel at concentrations of 5%, 10%, and 15%. The spray gel formulation was evaluated for its physical quality and SPF value.* ***Results****: The variation in extract concentration has a statistically significant effect on the physical properties of the preparation and SPF values (P<0.05). The physical stability conditions in each formula (F1, F2, and F3) meet the requirements of the spray gel preparation in terms of pH, viscosity, spreading test, drying time test, and adhesion test. The spray gel preparations F1 (5%), F2 (10%), F3 (15%) each have SPF values of 20, 25, and 35 respectively.* ***Conclusion****: The spray gel formulations in F1 (5%), F2 (10%), and F3 (15%) are physically stable and have moderate to high SPF values, with F3 (15%) having the highest SPF value of 35.*

Keywords*: Citrus aurantifolia, spray gel, topical sunscreen formulation*

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INTRODUCTION

Sun exposure has harmful effects on the skin due to UV radiation from UV-A and UV-B rays *(D'Orazio et al*., 2013). The human skin has natural defense mechanisms against sunlight, including sweating, melanin production, and thickening of the stratum corneum (Kalangi, 2014). Sunscreen formulations are commonly used to protect the skin by blocking UV rays (Dutra *et al*., 2004).

Lime peel contains over 60% flavonoids and has potential as a sunscreen agent (Pratiwi *et al*., 2017). The flavonoid compounds represent the principal secondary metabolites found in lime peel, belonging to the group of phenolic compounds capable of absorbing UV-A and UV-B rays from the sun. To confirm the UV absorption capability of lime peel, this study conducted a total phenol assay on the extract before it was formulated.(Andy Suryadi *et al*., 2021). Previous studies have demonstrated the sunscreen activity of lime peel extract at various concentrations, with SPF values ranging from 4.4 to 40.15 (Yasin, 2017). Gel, cream, and lotion formulations containing lime peel extract also showed sunscreen activity, with SPF values ranging from 11.36 to 20.68, 12.01 to 18.57, and 11.27 to 19.44, respectively (Kularti, 2019; Nafisah, 2019; Zuhroh, 2019). These findings highlight the potential of lime peel as a sunscreen agent.

To further explore the properties of lime peel extract-based sunscreen, research is needed on its physical characteristics, stability, and Sun Protection Factor (SPF). The study aims to develop a sunscreen spray gel formulation using lime peel extract (*Citrus aurantifolia*) as the active ingredient. The choice of a spray form is based on its ability to provide concentrated content that dries quickly, offering a convenient and pleasant user experience.

MATERIALS AND METHODS

Material

Lime (*Citrus aurantifolia*), filter paper, distilled water (Smart-Lab), absolute ethanol (Smart-Lab), Folin-Ciocalteau reagent, aquabides (Onemed), carbopol 940 (Newman Chemicals), HPMC, propylene glycol (DOW Chemical Pacific), methyl paraben (Salicylates and Chemicals), propyl paraben (Salicylates and Chemicals), triethanolamine (TEA) (Emplura), Na_2CO_3, and gallic acid (Sigma-Aldrich).

Tools

Oven (Memmert), grinder, dehydrator, hotplate (Philips), rotary evaporator (Heidolph), water bath (Memmert), furnace, calipers, analytical balance (Mettler Toledo), UV-Vis spectrophotometer (Shimadzu UV-1280), pH meter (Mettler Toledo), ultrasonic cleaner (Branson), viscometer (Brookfield LV), and magnetic stirrer (C-MAG HS 7 IKA).

Method

Preparation of simplisia

The lime samples used were initially determined at the Ecology and Biosystematics Laboratory, Biology Department, Faculty of Science and Mathematics, Diponegoro University, Semarang, to ensure that the lime used in the research is *Citrus aurantifolia*. The ripe lime fruits (*Citrus aurantifolia*), which were dark green in color, were sorted when wet, then thoroughly washed with running water to remove any dirt attached to them. Subsequently, the lime peels were separated from the fruit using a lime peeler. The herbal material was then dried using a dehydrator at a temperature of 50ºC for one week to obtain thoroughly dried herbal material. The dried herbal material was ground into a fine powder using a grinder.

Extraction

The extraction method used was maceration using 70% ethanol. A total of 1000.27 grams of powdered herbal material was soaked in a covered container with 10 liters of 70% ethanol solvent in a ratio of 1:10 (herbal material to solvent weight) for 24 hours, with 10 minutes of stirring each day. The maceration process was repeated three times using 5 liters of 70% ethanol solvent in a ratio of 1:5. The macerate was filtered using filter paper and then evaporated under a pressure of 75 mbar and a temperature of 50°C using a rotary evaporator. The resulting liquid extract was concentrated using a water bath at a temperature of 50°C until it thickened into a paste-like consistency (Zuhroh, 2019).

Determination of total phenolic content

Preparation of gallic acid stock solution

A total of 10 mg of gallic acid was dissolved in 10 mL of analytical grade ethanol to create a 1000 ppm gallic acid stock solution. Serial dilutions of the gallic acid stock solution were made to obtain final concentrations of 5, 10, 20, 30, and 40 ppm.

Preparation of 7.5% Na_2CO_3 solution

A total of 7,5 grams of Na_2CO_3 were weighed and dissolved in 100 mL of distilled water (Andriani & Murtisiwi, 2018).

Determination of operating time (OT)

A total of 300 µL of a 30 ppm gallic acid solution was mixed with 1.5 mL of Folin-Ciocalteau reagent, stirred, and left undisturbed for 3 minutes. Then, 1.2 mL of a 7.5% Na_2CO_3 solution was added, thoroughly

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mixed, and allowed to stand at room temperature throughout the operating time. The absorbance of the solution was measured at λ765 nm within the range of 0-60 minutes, and the point at which the solution reached a stable absorbance was determined as the operating time (Andriani & Murtisiwi, 2018).

Determination of maximum wavelength

A total of 300 µL of a 30 ppm gallic acid solution was mixed with 1.5 mL of Folin-Ciocalteau reagent, stirred, and left undisturbed for 3 minutes. Then, 1.2 mL of 7.5% Na_2CO_3 solution was added, thoroughly mixed, and allowed to stand for the operating time (30 minutes) at room temperature. The absorbance of the solution was measured within the wavelength range of 600-850 nm to determine the maximum wavelength (Andriani & Murtisiwi, 2018).

Measurement of standard gallic acid solutions

A total of 300 µL of each solution with concentrations of 5, 10, 20, 30, and 40 ppm was taken and mixed with 1.5 mL of Folin-Ciocalteau reagent. The mixture was stirred and left for 3 minutes. Then, 1.2 mL of a 7.5% Na_2CO_3 solution was added, and the solution was thoroughly mixed until homogenous. It was then left at room temperature for the operating time (30 minutes). The absorbance of the solution was measured at the gallic acid maximum wavelength. A gallic acid calibration curve was constructed based on the measured absorbance (Andriani & Murtisiwi, 2018).

Determination of total phenolic content

The lime peel extract was prepared at a concentration of 1000 ppm by weighing 10 mg of the extract and dissolving it in 10 mL of analytical grade ethanol. The extract solution was further diluted with analytical grade ethanol to a concentration of 100 ppm. Then, 300 µL of the diluted extract solution was mixed with 1.5 mL of Folin-Ciocalteau reagent, shaken, and left to stand for 3 minutes. After that, 1.2 mL of 7.5% Na_2CO_3 solution was added, followed by thorough mixing and incubation at the operating time (30 minutes). The absorbance was measured at the maximum wavelength (745.8 nm). The obtained phenolic content was recorded as the mg equivalent of gallic acid per gram of sample. The measurement was performed three times, and the total phenolic content was calculated using the appropriate formula (Andriani & Murtisiwi, 2018).

$$\text{Total Phenolic Content} = \frac{\text{C x V x FP}}{\text{g}}$$

Note: C = concentration (mg/mL; V = volume of extract (ml); FP = dilution factor; g = the weight of sample used (gram)

Formulation of spray gel preparation

The formulation of lime peel extract spray gel can be seen in Table 1. Methyl paraben and propyl paraben were dissolved in propylene glycol. Carbopol was dispersed in hot distilled water until homogeneous, then triethanolamine was added, and the mixture was homogenized with a combination of methyl paraben. HPMC was gradually dispersed into a beaker containing hot distilled water and stirred until homogeneous. The carbopol mixture was poured into the HPMC and sonicated until a homogeneous solution was obtained. Lime peel extract was dispersed in distilled water and sonicated until a homogeneous extract solution was obtained. The extract was added to the HPMC and carbopol mixture, followed by the addition of distilled water to a total volume of 100 mL, and sonicated for 5 minutes. The preparation was filled into spray containers (Suyudi, 2014).

Testing of physical properties of the preparation

The physical properties of lime peel (*Citrus aurantifolia*) spray gel formulation were tested using the following methods for each observed formula with 3 replicates.

Table 1. Formula of Sunscreen Spray Gel with Lime Peel Extract (*Citrus aurantifolia*)

Materials	Function of Materials	Concentration of Materials (*b/v* %)			
		K-*	F1	F2	F3
Lime peel extract	Active ingredient	-	5	10	15
Karbopol 940	Gelling agent	1	1	1	1
HPMC		2	2	2	2
Propylene Glycol	Humectant	15	15	15	15
Methyl paraben	Preservative	0.18	0.18	0.18	0.18
Propyl paraben		0.02	0.02	0.02	0.02
Triethanolamine	Alkalizing agent	qs	qs	qs	qs
Distilled water ad	Solvent	100	100	100	100

*K- = Negative Control (without addition of lime peel extract)

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Organoleptic test

Organoleptic observations were conducted by visually assessing the appearance of the formulation, including color, odor, clarity, homogeneity, separation, and any other changes that may occur after preparation (Depkes RI, 2020).

pH test

The pH of the spray gel formulation was measured using a pH meter. pH examination was performed to ensure that the pH value of the formulation falls within the required range for topical preparations (4.5-6.5) to prevent irritation (Depkes RI, 2020).

Viscosity test

A sample of the formulation (100 mL) was taken and placed in a Brookfield viscometer with spindle number 61 at a speed of 12 rpm. The viscosity reading was recorded once the value on the viscometer stabilized (Depkes RI, 2020).

Spreadability test

The formulation was sprayed onto a plastic film at a distance of 5 cm, and the spreadability of the formulation was measured using a caliper. The parameter used for measurement was the diameter (Depkes RI, 2020).

Drying time test

The formulation was sprayed onto the inner forearm of a volunteer at a distance of 5 cm. The time required for the formulation to dry was measured using a stopwatch and recorded (Hayati, R. et al., 2019).

Adhesion test

For adhesion testing, the formulation was applied to the inner side of the lower arm of a volunteer by spraying it at a distance of 5 cm. If the spray gel droplets dripped within 10 seconds, it was evaluated as dripping; if the droplets did not drip within 10 seconds, it was evaluated as adhering (Depkes RI, 2020)..

Stability testing of the preparation

The preparation was placed at a cold temperature (4±2°C) for 24 hours, followed by exposure to a hot temperature (40±2°C) for 24 hours (1 cycle). The testing was performed for 6 cycles, and the physical changes of the spray gel preparation were observed at the beginning and end of each cycle, including organoleptic evaluation, pH measurement, viscosity determination, spreading ability, drying time, and adhesive properties

SPF value testing of the preparation

The determination of the SPF value of lime peel extract begins with weighing each formulation, including F1 (5%), F2 (10%), F3 (15%), positive controls (NIVEA® sunscreen spray SPF 30, Wardah® UV Shield Essential Sunscreen Gel SPF 30, and Emina® Sun Battle SPF 30), and negative control (formulation without extract), amounting to 1 gram. The correction factor (CF) is determined by measuring the absorbance of the positive controls, which have known SPF values. Each weighted formulation is combined with 50 mL of 70% ethanol and sonicated for 15 minutes. The sonicated formulation is transferred to a 100 mL volumetric flask and filled with 70% ethanol up to the mark. The formulation is then filtered using filter paper, and the first 10 mL of the filtrate is discarded. An aliquot (filtered formulation) of 100 µL is pipetted into a 25 mL volumetric flask and diluted with 70% ethanol up to the mark. Subsequently, the absorbance is measured using a UV-Vis spectrophotometer. The absorbance spectrum of the sample in solution form is obtained at wavelengths ranging from 290 to 320 nm with a 5 nm interval, using 70% ethanol as the blank. The absorbance values for each concentration are recorded and used to calculate the SPF value (Dutra et al., 2004). The SPF calculation according to the Mansur equation is as follows, with EE x I representing a constant factor.

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Note: CF= Correction Factor; EE= Erythema Effect; I= Intensity of sunlight; abs = sample absorbance

Data Analysis

The physical test data and SPF values of the preparations were analyzed using One-way ANOVA with a significance level of $p<0.05$. If the significance value is <0.05, then the test is continued with a post-hoc test. Additionally, a paired t-test was conducted with a significance level of $p<0.05$ to assess the physical stability of the preparations by comparing the physical conditions at cycles 0 and 6.

RESULTS AND DISCUSSION

Extraction

The extraction in this study was performed using the maceration method, which is simple, does not involve heating, and does not require special equipment. The material was soaked to break the cell walls and membranes through a pressure difference. Secondary metabolites in the cytoplasm are dissolved in the organic solvent (Ditjen POM, 2000). The extraction of phenolic compounds was conducted using a mixture of 70% ethanol and water. Ethanol 70% has the appropriate polarity for extracting flavonoids and tannins. Additionally, it has low toxicity and readily evaporates (Djarot et al., 2019). Stirring was performed to achieve concentration equilibrium. The re-maceration process was employed to extract any remaining compounds in

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the residue after solvent saturation (Andriani & Murtisiwi, 2018). The filtrate was concentrated using a water bath at 50°C, resulting in a concentrated extract weighing 185.9 grams with a yield of 18.58%. The yield value is related to the content of bioactive compounds in the raw material. A higher yield corresponds to a higher desired substance content (Ditjen POM, 2000).

Determination of total phenolic content

The determination of total phenolic content was performed using the Folin-Ciocalteau reagent. Phenolic compounds can react with this reagent to form a solution with measurable absorbance. The Folin-Ciocalteau reagent oxidizes the hydroxyl groups of phenolic compounds, forming a blue-colored complex. This reaction proceeds slowly under acidic conditions, Na_2CO_3 was added during the test to create a basic environment and accelerate the reaction (Andriani & Murtisiwi, 2018).

The standard solution used was gallic acid, which is a simple, natural, and stable phenolic compound. During the reaction, the hydroxyl groups in the phenolic compounds react with the Folin-Ciocalteau reagent, forming a blue-colored molybdenum-tungsten complex. The intensity of the blue color increases with the concentration of phenolate ions formed. In other words, the higher the concentration of phenolic compounds, the more phenolate ions will reduce the heteropoly acid (phosphomolybdate-phosphotungstate) to form the molybdenum-tungsten complex, resulting in a darker color (Andriani & Murtisiwi, 2018).

The absorbance measurements of the gallic acid standard solution were used to construct a calibration curve. The curve, shown in Figure 1, follows a linear equation y = 0.0111x + 0.2391 with a correlation coefficient (R) of 0.9778. This curve was used to determine the phenolic content of the sample. The average total phenolic content of lime peel extract was 34.8845 ± 0.6511 mg GAE/g, indicating that each gram of lime peel extract is equivalent to 34.8845 mg of flavonoid.

Testing of physical properties of the preparation

To achieve good and acceptable pharmaceutical formulations in society, the physical properties and stability of the preparations must be examined. Physical properties serve as determinants of the quality of pharmaceutical preparations. The physical characterization tests include organoleptic evaluation, pH, viscosity, spreadability, drying time, and adhesion. The statistical analysis results for the pH, viscosity, spreadability, drying time, and adhesion tests showed $p > 0.05$ in the Shapiro-Wilk test of normality, indicating that the data are normally distributed. Levene's test results also showed $p > 0.05$, indicating that the data are homogeneously distributed. In the One-Way ANOVA analysis, a p-value of less than 0.05 was obtained, indicating that the variation in lime peel extract concentrations has a statistically significant effect on the tested physical properties. Subsequently, a post-hoc analysis was conducted to examine the differences in the tested physical property values among the different formulas. The test results revealed a p-value of less than 0.05, indicating that there are statistically significant differences in pH, viscosity, spreadability, drying time, and adhesion values among the different concentrations of the extract. The results of physical property tests for the three formulas can be observed in Tables 2 and 3.

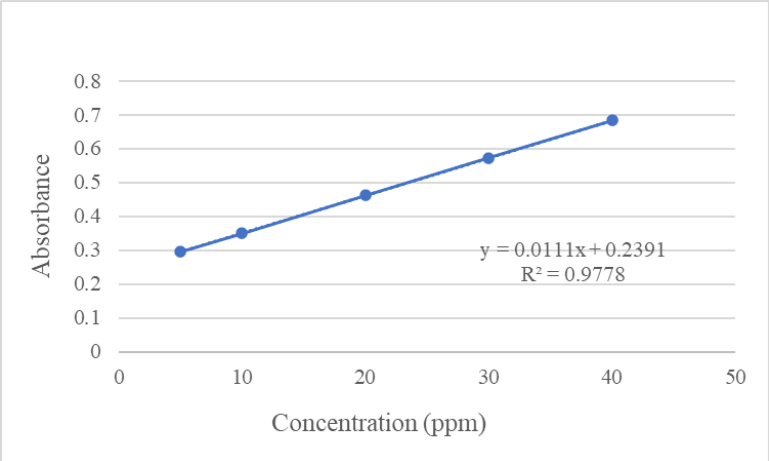

Figure 1. Gallic Acid Standard Curve

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Tabel 2. Organoleptic test results of sunscreen spray gel formulation with lime peel extract

Formula	Organoleptic			
	Form	**Odor**	**Color**	**Homogeneity**
F1 (5%)	Liquid	Lime smell	Yellow – brown	Homogeneous
F2 (10%)	Liquid	Lime smell	Brown	Homogeneous
F3 (15%)	Liquid	Lime smell	Dark brown	Homogeneous

Table 3. Results and significance test of sunscreen spray gel formulation with lime peel extract

Test	Formula	Results ± SD	Requirement	Sig. ANOVA ($p < 0.05$)
pH Test	F1 (5%)	5.3 ± 0.03	4.5 – 6.5	0.000
	F2 (10%)	5.2 ± 0.05		
	F3 (15%)	5.0 ± 0.05		
Viscosity Test	F1 (5%)	82.74 ± 1.57	< 150 cP	0.000
	F2 (10%)	112.07 ± 1.40		
	F3 (15%)	131.28 ± 1.78		
Spreadibility Test	F1 (5%)	6.4 ± 0.03	5 -7 cm	0.000
	F2 (10%)	5.8 ± 0.07		
	F3 (15%)	5.3 ± 0.07		
Drying Time Test	F1 (5%)	1.3 ± 0.08	< 5 minutes	0.000
	F2 (10%)	2.5 ± 0.06		
	F3 (15%)	3.4 ± 0.11		
Adhesion Test	F1 (5%)	36 ± 1	> 10 seconds	0.000
	F2 (10%)	61 ± 2		
	F3 (15%)	82 ± 2		

Organoleptic test

The three formulas have the same color and homogeneity, but they differ in consistency and color. A good formulation is characterized by a pleasant odor, attractive color, good consistency, and homogeneity. The consistency of the spray gel preparation is in liquid form, in accordance with its definition, which is one form of gel formulation development, which is a water-based phase system comprising at least 10% to 90% of the formulation's weight. The term 'spray' is defined as a composition that can be dispensed from its applicator, such as an aerosol or spray pump. A homogeneous formulation refers to a preparation that does not contain coarse particles, has evenly dispersed particles, and has a uniform color (Salwa et al., 2020). Although the preparation is in liquid form, the consistency of each formula is different. Formula III has the thickest consistency because it has the highest concentration of lime peel extract, which is 15%. This result proves that the higher the concentration of the extract, the thicker the resulting formulation, with a more intense color pigmentation.

pH test

The obtained results indicate that an increase in the concentration of lime peel extract has an effect on the pH value of the formulation, causing it to decrease. This is due to the higher concentration of salicylic acid, amino acids, citric acid, and vitamin C in the lime peel extract. As a result, the pH value of the formulation decreases. All formulations have met the requirement for a good pH value, which is in line with the pH of the skin ranges from 4,5 to 6,5. If the spray gel formulation is too acidic, it may cause skin irritation. On the other hand, if the pH of the formulation is too alkaline, it may lead to dryness of the skin (Hayati, R. et al., 2019).

Viscosity test

The obtained results indicate that an increase in the concentration of lime peel extract affects the viscosity value of the formulation, leading to an increase in viscosity. This is because higher concentrations of lime peel extract result in a thicker formulation. Viscosity also influences the spreadibility, drying time, and adhesion of the resulting formulation (Lachman et al., 2008). All formulations have met the requirement for a good viscosity value for the spray gel formulation, which is below 150 cP (Hayati, R. et al., 2019).

Spreadibility test

The results obtained indicate that as the concentration of lime peel extract increases, the spreadibility value of the formulation decreases. This is because higher concentrations of lime peel extract lead to a thicker formulation, reducing its ability to spread. Consequently, the opportunity for the active ingredients to come into contact with the skin diminishes, resulting in a decrease in the effectiveness of the formulation when applied topically (Jawa La et al., 2020). All

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formulations demonstrated a spreading pattern when sprayed and met the requirement for an ideal spreadibility value for the spray gel formulation, which is 5-7cm (Depkes RI, 2020).

Drying time test

The results obtained indicate that as the concentration of lime peel extract increases, the drying time of the formulation also increases. This is because higher concentrations of lime peel extract result in a thicker formulation, which requires more time to dry. All formulations have met the requirement for a good drying time value for the spray gel formulation, which is less than 5 minutes to prevent stickiness on the skin and provide comfort for the consumer when applied (Hayati, R. et al., 2019).

Adhesion test

The results obtained indicate that as the concentration of lime peel extract increases, the adhesion value of the formulation also increases. This is because higher concentrations of lime peel extract result in a thicker formulation, leading to a longer adhesion time and increased release of active ingredients. A sunscreen formulation is expected to adhere to the skin for a longer period of time to provide prolonged protection against ultraviolet radiation (Hana Shovyana & Karim Zulkarnain, 2013). All formulations can be considered to adhere well to the skin as long as the formulation droplets do not drip from the skin within less than 10 seconds (Hayati, R. et al., 2019).

Stability testing of the preparation

The entire sunscreen spray gel formula is stored at a cold temperature of 4°C ± 2°C for 24 hours and at a high temperature of 40°C ± 2°C for 24 hours (1 cycle). After that, a physical stability test is conducted for 6 cycles. The results of the physical stability test for the sunscreen spray gel formulation can be seen in Tables 4 and 5.

The statistical analysis results indicate that if the p-value is greater than 0.05 in the paired t-test, there is no significant difference or physical stability in the tested sample. However, if the p-value is less than 0.05 in the paired t-test, it indicates a significant difference or physical instability in the tested sample.

Tabel 4. Organoleptic stability test results of sunscreen spray gel formulation with lime peel extract

Formula	Cycle	Organoleptic			
		Form	Odor	Color	Homogeneity
F1 (5%)	0	Liquid	Lime smell	Yellow – brown	Homogeneous
	6	Liquid	Lime smell	Yellow – brown	Homogeneous
F2 (10%)	0	Liquid	Lime smell	Brown	Homogeneous
	6	Liquid	Lime smell	Brown	Homogeneous
F3 (15%)	0	Liquid	Lime smell	Dark brown	Homogeneous
	6	Liquid	Lime smell	Dark brown	Homogeneous

Table 5. Results and significance of the physical stability test for the sunscreen spray gel formulation

Test	Formula	Results ± SD		Requirement	Sig (2-tailed) (p < 0.05)
		Cycle 0	Cycle 6		
pH Test	F1 (5%)	5.3 ± 0.03	5.3 ± 0.02		0.053
	F2 (10%)	5.2 ± 0.05	5.1 ± 0.05	4.5 – 6.5	0.095
	F3 (15%)	5.0 ± 0.05	4.9 ± 0.06		0.057
Viscosity Test	F1 (5%)	82.74 ± 1.57	81,96 ± 1.52		0.003
	F2 (10%)	112.07 ± 1.40	111.30 ± 1.59	< 150 cP	0.020
	F3 (15%)	131.28 ± 1.78	130.04 ± 1.71		0.004
Spreadibility Test	F1 (5%)	6.4 ± 0.03	6.4 ± 0.03		0.015
	F2 (10%)	5.8 ± 0.07	5.9 ± 0.09	5 -7 cm	0.020
	F3 (15%)	5.3 ± 0.07	5.4 ± 0.05		0.044
Drying Time Test	F1 (5%)	1.3 ± 0.08	1.2 ± 0.07		0.011
	F2 (10%)	2.5 ± 0.06	2.5 ± 0.05	< 5 minutes	0.006
	F3 (15%)	3.4 ± 0.11	3.3 ± 0.11		0.005
Adhesion Test	F1 (5%)	36 ± 1	32 ± 2		0.024
	F2 (10%)	61 ± 2	53 ± 2	> 10 seconds	0.001
	F3 (15%)	82 ± 2	73 ± 2		0.015

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Table 6. Results of correction factor (CF) for positive control of sunscreen formulation

Positive Control	SPF	Correction Factor (CF)	Results ± SD
NIVEA®	30	46.96	
Wardah®	30	49.28	46.49 ± 3.05
Emina®	30	43.24	

Organoleptic test

The organoleptic properties observed in the formulation include the form and consistency, color, odor, and homogeneity of the spray gel. The results of the organoleptic testing of sunscreen spray gel formulations F1, F2, and F3 indicate that there were no changes in odor, color, and form of the formulation, and no visible phase separation throughout the 6 cycles of storage, both at cold and high temperatures. This indicates that the spray gel formulation exhibits good stability in organoleptic tests over the 6-cycle storage period.

pH test

The obtained results indicate that the formulation is stable, but there is a decrease in pH during the stability test. Changes in pH values during storage indicate reactions or damage to the components within the formulation, resulting in an increase or decrease in pH value (Barel et al., 2009). This can occur due to oxidation reactions on the carboxylic acid groups of the acid compound in the extract, leading to the addition of hydrogen atoms and a decrease in pH value. Additionally, the use of transparent packaging is another factor contributing to the instability of the pH in the formulation as it allows light to interact and cause degradation reactions of secondary metabolites in the formulation (Tranggono & Latifah, 2007). This can be addressed by storing the formulation in a place that is not exposed to light and at an appropriate temperature. The choice of packaging should be tailored to the properties of the active substance and should protect the product from external influences. The use of buffers is also necessary in the formula to maintain the stability of the pH.

Viscosity test

The obtained results indicate that the formulation is physically unstable in terms of viscosity, but it still meets the viscosity acceptance criteria for a spray gel. The decrease in viscosity can be attributed to storing the formulation at high temperatures, which causes the active molecules in the formulation to move, weakening the intermolecular interactions and resulting in a decrease in viscosity (Putra et al., 2014). Choosing an ideal storage temperature is important to maintain the viscosity stability of the formulation. Stability testing of the viscosity of the spray gel is crucial to ensuring that the formulation remains easy to spray through the applicator and adheres to the skin.

Spreadibility test

The obtained results indicate that the formulation is physically unstable in terms of spreading power, but it still meets the acceptance criteria for spreading power in a spray gel. This is due to a decrease in viscosity after storage, resulting in the weakening of the gel matrix's strength in the formulation, which leads to an increase in the spreading power of the formulation (Putra et al., 2014).

Drying time test

The obtained results indicate that the formulation is physically unstable in terms of drying time, but it still meets the acceptance criteria for drying time in a spray gel. This is due to a decrease in viscosity after storage, resulting in the formulation becoming more watery, which leads to a faster drying time (Hayati, R. et al., 2019).

Adhesion test

The obtained results indicate that the formulation is physically unstable in terms of adhesion power, but it still meets the acceptance criteria for adhesion power in a spray gel. This is due to a decrease in viscosity after storage, resulting in the formulation becoming more watery, which leads to a decrease in the adhesion power of the formulation (Hayati, R. et al., 2019).

SPF value testing of the preparation

The determination of the correction factor (CF) value in this study was done by measuring the absorbance of sunscreen products with known SPF values to ensure the calculation of SPF based on the formula. The absorbance values were then processed using the Mansur equation to determine the CF value used to account for the spectrophotometry and solvent usage (Allen & Ansel, 2014). The positive control sunscreen products used in this study included NIVEA® sunscreen spray SPF 30, Wardah® UV Shield Essential Sunscreen Gel SPF 30, and Emina® Sun Battle SPF 30. The results of the Correction Factor (CF) for the Positive Control of the Sunscreen Formulation can be seen in Table 6.

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Table 7. Results and significance of spf testing for the spray gel

Formula	Results ± SD	SPF Categories	Sig. ANOVA (p < 0.05)
F1 (5%)	20 ± 0.2	Medium	
F2 (10%)	25 ± 0.4	Medium	0.000
F3 (15%)	35 ± 0.1	High	

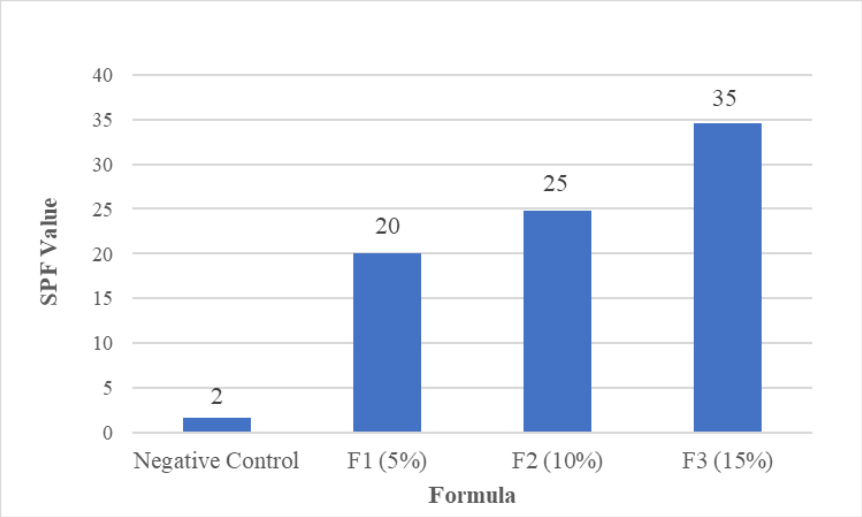

Figure 2. Graph of variation in extract concentration against spf value

The selection of three different products with known SPF values as positive controls aimed to validate the chosen method for this study. The average CF value obtained from these three products was 46.4945. This CF value would then be used to calculate the SPF value of the samples tested in this study. The SPF value testing on the negative control (formulation without extract) yielded an SPF value of 1,6687. The SPF value of the negative control indicated that the polymer in the gel spray without extract had no significant effect on the SPF value of the resulting gel spray formulation. The results of the SPF value testing for the gel spray formulation can be seen in Table 7, along with the graph showing the variation of extract concentration on the SPF value in Figure 2.

In accordance with the data presented in Table 7, the SPF values derived from the three formulations are classified within the medium to high range. This classification is established according to the protection range defined by the Indonesian Food and Drug Monitoring Agency (BPOM). SPF values within the range of ⩾6 - <15 are categorized as low, ⩾15 - <30 as moderate, ⩾ 30 - <50 as high, and ⩾ 50 are classified as very high (BPOM RI, 2020). F1 and F2 belonged to the moderate protection category against UV rays, while F3 belonged to the high protection category. Increasing the extract concentration enhanced the SPF value in the sunscreen gel formulation due to the higher phenolic compound content in the formulation (Zuhroh, 2019). The sunscreen activity of the formulation was attributed to the presence of phenolic compounds in the lime peel extract, which had conjugated aromatic benzene groups capable of absorbing UV-B rays that can be harmful to the skin. Higher SPF values indicate longer protection against UV rays (Dutra et al., 2004).

The statistical analysis showed a p-value > 0.05 in the Shapiro-Wilk normality test, indicating a normal data distribution. Levene's test yielded a p-value > 0.05, indicating a homogeneous distribution of data. The One-Way ANOVA analysis resulted in a p-value < 0.05, indicating that the variation in lime peel extract concentrations significantly affected the SPF values of the formulations. Further post-hoc statistical analysis was conducted to determine the differences in SPF values between each formula. The test results obtained a p-value <0.05, indicating statistically that each concentration of the extract in every formula produces significantly different SPF values. This is because higher extract concentrations result in higher SPF values for the formulation.

To achieve the desired sun protection factor (SPF) value, the sunscreen gel spray formulation should be applied evenly at a rate of 2 mg/cm^2 (Diffey, B., 2000). The average surface area of the human face is approximately 3.5% of the total skin surface area (Liu, Y. et al., 2008). Therefore, an estimated 1.12 grams of

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sunscreen are needed to cover the entire facial surface. The spray gel formulation for each formula is then weighed to determine the appropriate volume, and a spray test is conducted at a distance of 20 cm, resulting in 3-4 sprays to achieve the sunscreen dosage corresponding to the SPF value on the potentially exposed skin area.

CONCLUSION

The conclusion of this study is that the variation in the concentration of lime peel extract affects the physical properties and SPF value of the sunscreen gel spray formulation with a value of $p < 0.05$. Each formulation of the spray gel demonstrates good physical stability in viscosity, spreadibility, drying time, and adhesion tests. The SPF values derived from F1(5%), F2 (10%) dan F3 (15%) are classified within the medium to high range, with F3 (15%) having the highest SPF value of 34.64, classified as providing high protection against UV-rays.

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AUTHOR CONTRIBUTIONS

Conceptualization, E.R.S., F.I.W., E.V.R.; Methodology, E.R.S., F.I.W., E.V.R.; Software, E.R.S., F.I.W., E.V.R.; Validation, E.R.S., F.I.W., E.V.R.; Formal Analysis, E.R.S., F.I.W., E.V.R.; Investigation, E.R.S., F.I.W., E.V.R.; Resources, E.R.S., F.I.W., E.V.R.; Data Curation, E.R.S., F.I.W., E.V.R.; Writing - Original Draft, E.R.S., F.I.W., E.V.R.; Writing - Review & Editing, E.R.S., F.I.W., E.V.R.; Visualization, E.R.S., F.I.W., E.V.R.; Supervision, E.R.S., F.I.W., E.V.R.; Project Administration, E.R.S., F.I.W., E.V.R.; Funding Acquisition, E.R.S., F.I.W., E.V.R.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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