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Research Article

Determination of Sun Protection Factor Value and Physical Stability of Wungu Leaf (*Graptophylum pictum* (L.) Griff) Extract Spray Sunscreen

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ABSTRACT

Sunscreen can protect the skin from ultraviolet (UV) radiation. The inorganic active ingredients in sunscreens can have detrimental effects on the skin. Wungu leaf (*Graptophylum pictum* (L.) Griff) is a suitable alternative due to its antioxidant activity and similar mechanism of action to other antioxidants with lower side effects. This study employed a true experimental method, and the data were analyzed using one-way ANOVA and T-tests. The effectiveness of sunscreen is determined by the Sun Protection Factor (SPF) value. This study aims to determine the SPF value and physical stability of Wungu leaf extract spray sunscreen at concentrations of 500 ppm, 1,000 ppm, and 1,500 ppm. The results show that the spray sunscreens provide ultra-protection. The difference in extract concentration significantly affected the SPF value ($p<0.05$). Stability tests showed a negligible effect on color variation and a notable effect on pH and spray diameter. Despite its noticeable impact, the outcome remains within an acceptable range. The study concluded that the Wungu leaf extract spray sunscreen provides excellent photoprotection, as demonstrated by the high SPF values observed for Formula 1 (18.63), Formula 2 (23.87) and Formula 3 (29.56). In addition, the product exhibits robust physical stability, as assessed by relevant parameters.

INTRODUCTION

Ultraviolet (UV) radiation is energy produced by the sun in the 100-400 nm wavelength range. Although UV can benefit the skin, it can damage it by forming Reactive Oxygen Species (ROS). Human skin has natural protection against UV radiation but also requires external photoprotective agents to optimize the protective effect, and sunscreen is one such agent (Minerva, 2019; Wadoe *et al.*, 2020).

Sunscreen is a cosmetic that protects the skin from UV radiation (Yanti Eff *et al.*, 2019). Inorganic active ingredients such as titanium dioxide (TiO2) are still commonly found in sunscreens, even though the International Agency for Research on Cancer (IARC) classifies this compound as a carcinogenic substance (IARC, 2010). Based on these problems, an alternative active ingredient is needed to minimize the side effects caused. Wungu leaf (*Graptophylum pictum* (L.) Griff.) is a suitable alternative because it is proven to have antioxidant activity.

The content of flavonoids in Wungu leaf is proven to have antioxidant activity (Rustini and Ariati, 2017; Tandi *et al.*, 2021). All of these plants have antioxidant activity, but the dominant content is found in the leaves (Indrawati *et al.*, 2022). The antioxidant effects of Wungu leaf can be classified as moderate to strongest, depending on the fractions and differences in concentrations used (Salim and Suryani, 2020). Poh-Yen *et al.* (2018) explained that Wungu leaf extract with an ethanol concentration of 200 ppm could protect against UV radiation. The mechanism of antioxidant action by Wungu leaf is almost the same as the antioxidant effect produced by titanium dioxide. The mechanism of action of the flavonoid content in Wungu leaf works by reducing oxidative stress

(ROS) (Iova *et al*., 2021). Flavonoid compounds will inhibit the process of ROS formation by breaking the chain reaction of these free radicals. The mechanism of action that occurs between flavonoid compounds and free radicals is that the free hydroxyl groups in the flavonoid compound group will donate electrons, and free radical molecules will then replace them. This process causes flavonoid compounds to become free radicals but has a lower level of toxicity and is quickly neutralized by other antioxidants (Sánchez *et al*., 2019; Banjarnahor and Artanti, 2014).

Most sunscreens are formulated in creams and lotions, which have the drawback of leaving an oily feeling after application. These issues may seem trivial, but in the long run they can be very inconvenient for consumers. In response to this problem, this study formulated sunscreens in spray form, making them more convenient to apply.

The effectiveness of sunscreen is measured by the Sun Protection Factor (SPF) value and can be determined using UV-Vis spectrophotometry (Pratama and Zulkarnain, 2015). Each concentration must be tested on sunscreen preparations because different concentrations of active ingredients can affect the resulting SPF value. The quality of spray sunscreens is not only determined by their photoprotective effect but also by their physical stability.

Based on these reasons, this research was conducted to determine the physical stability and the SPF value of Wungu leaf extract spray sunscreen in vitro at concentrations of 500 ppm, 1,000 ppm, and 1,500 ppm.

METHODS

Materials and Tools

The material used in this study was the dry extract of Wungu leaf (*Graptophyllum pictum* (L.) Griff) from Karanganyar, Central Java. Methylparaben (Bratachem, food grade), Propylparaben (Bratachem, food grade), Glycerin (Bratachem, food grade), Propylene Glycol (Bratachem, food grade), BHT (Bratachem, food grade), Carbopol 940 (Bratachem, food grade), Triethanolamine (TEA) (Bratachem, food grade), Green Dye, and aquadestilata.

The tools used in this study were UV-Vis spectrophotometer (Shimadzu UV-1900i), rotary evaporator (Heidolph Hei-VAP Expert), analytical balance (Shimadzu AT-R Series), oven (Memmert Universal Oven Um), refrigerator (Sharp SJ-N162N-HS), Ostwald viscometer, pH meter (Mettler Toledo SevenExcellence pH meter S400-Std-Kit), magnetic stirrer, glassware (Pyrex, Iwaki, and Herma), laboratory spatula, porcelain cup, filter cloth, aluminum foil (Klin pak), tripod, bunsen, asbestos gauze, and spray bottle.

Preparation and Analysis of Sample

Wungu leaf dry powder was extracted using the maceration method in 70% ethanol. The extract obtained was then concentrated using a rotary evaporator until thick. The thick extract obtained was then dried using an oven until dry. The extract was evaluated and identified by Materia Medica Batu, East Java.

Formula of Spray Sunscreen

Table 1. *Daun Wungu* extract spray sunscreen formula

Materials	Function	Concentration		
		Formula 1	Formula 2	Formula 3
Daun Wungu extract	Active ingredient	50 mg	100 mg	150 mg
Prophyl Paraben	Preservative	0.014 g	0.014 g	0.014 g
Methyl Paraben	Preservative	0.027 g	0.027 g	0.027 g
Glyserin	Humectant	15 mL	15 mL	15 mL
Carbopol 940	Thickening agent	0.06 g	0.06 g	0.06 g
TEA	Alkalizing agent	0.05 mL	0.05 mL	0.05 mL
Prophylon Glycol	Cosolvent	10 mL	10 mL	10 mL
Green dye	Colorant	0.01 mL	0.01 mL	0.01 mL
Aquadest	Solvent	Ad 100 mL	Ad 100 mL	Ad 100 mL

The procedure for the formulation of spray sunscreen preparations from Wungu leaf extract follows Good Manufacturing Practice (GMP). Materials were prepared according to the formula (Table 1), then weighed. Carbopol 940 was dispersed in 1/3 distilled water that was heated to 70 °C for 30 minutes and stirred until homogeneous. TEA was added to the mixture and stirred until homogeneous (Mixture A). 1/3 aquadest was heated to 70 °C, and methylparaben, glycerin, propylene glycol, BHT, and propylparaben were added. Then the mixture was stirred until homogeneous (Mixture B). Mixture B was mixed with mixture A and stirred until homogeneous. Wungu leaf extract was dissolved first with ethanol 70% and then added to the preparation mixture. After that, the preparation was homogenized with a magnetic stirrer at a speed of 1200 rpm for 2 minutes. The homogeneous preparation was put into a bottle. Each formula was repeated for 3 repetitions with variations in the extract concentration for each formula was 500 ppm, 1,000 ppm, and 1,500 ppm.

Physical Stability Test

The physical stability test aims to see the physical stability of the preparations formulated using the temperature cycling test method for 6 cycles. For each cycle, the preparation will be placed at a temperature of 40±2 °C and 4±2 °C for 24 hours, respectively. In this test, the parameters observed were organoleptic (color, odor, texture, and clarity), homogeneity, pH, viscosity, and spray pattern. Observations on the stability test were performed by comparing the presence or absence of changes between the 0th and 6th cycles. Spray sunscreen preparations are considered to have good stability if, after the 6th cycle of the stability test, there is no significant change in the test parameters (Puspita *et al.*, 2021).

1. Organoleptic and Homogenity Test

The organoleptic tests are done by visual observation of the preparations between the 0th and 6th cycles (Salwa *et al.*, 2020). Parameters observed in this test were color, odor, texture, and clarity. The homogeneity test aims to observe the distribution of insoluble particles (Anindhita and Oktaviani, 2020; Salwa *et al.*, 2020). The preparation is considered to have good physical stability if, after a cycling test for 6 cycles, the homogeneity of the preparation is maintained, indicated by the absence of insoluble particles in the spray sunscreen preparation.

2. pH Test

The pH test aims to measure the acidity degree of the preparation. The safe pH range for topical preparations is 3.5-7 (Dayan, 2017; Luki´c *et al.*, 2021; Ariyanti *et al.*, 2021). The preparation is considered to have a stable pH if, after a cycle test for 6 cycles, no significant change is found in the pH of the spray sunscreen preparation (Puspita *et al.*, 2021). The pH test in this study used a pH meter (Mettler Toledo SevenExcellence pH meter S400-Std-Kit).

3. Viscosity Test

The viscosity test aims to measure the viscosity of the preparation using a viscometer. A viscosity test measured the density and flowed time of each preparation and distilled water using a pycnometer and Ostwald viscometer. Density is calculated using equation (i). The flow time is calculated using equation (ii).

$$\rho = \frac{m}{v} \quad \text{...... (i)}$$

Information:

ρ : Density of the preparation
m : Weight of the preparation
v : Volume of the pycnometer

$$\eta = \eta_o \frac{t.\rho}{t_o.\rho_o} \quad \text{....... (ii)}$$

Information:

η : Viscosity of the preparation (Ns/m^2)
ηo : Viscosity of water
t : Stock flow time
to : Water flow time
ρ : Density of the preparation
ρo : Density of water

The preparation is considered to have an appropriate viscosity if, after a cycle test, no significant change in the viscosity of the preparation is found, and it remains within the appropriate range. Good viscosity values for spray sunscreen preparations range from 500-5000 cPs (Rasyadi, 2018; Anindhita and Oktaviani, 2020; Salwa *et al.*, 2020).

4. Spray Pattern Test

The spray pattern test was conducted by spraying the preparation on mica plastic (Distances 3 cm, 5 cm, 10 cm, and 15 cm) for 3 repetitions, after which the diameter formed was calculated (Anindhita and Oktaviani, 2020). Spray sunscreen preparations are considered to have good physical stability if, after a stability test for 6 test cycles, there is no significant difference in the diameter of the spray pattern formed.

5. Determination of SPF Value

The SPF value of the spray sunscreen preparation was measured in vitro using a UV-Vis spectrophotometer. This instrument measures the wavelength and intensity of samples exposed to UV light (180-380 nm). The absorption of radiation exposure to the sample will cause electrons to move from the ground state to a higher energy level (chromophores) (Sharma *et al.*, 2020). The spectrophotometer will read this displacement process in the form of a spectrum translated into absorbance. This absorbance value is then entered into the Mansur equation to calculate the SPF value. Mansur's equation in Dutra *et al.* (2004) is as follows:

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

Information:
CF : Correction factor
EE : Spectrum of erythema effect
I : Spectrum of solar intensity
Abs : Sunscreen sample absorbance

The measured SPF value will then be compared with the Food and Drug Administration (FDA) classification. The classification compares the SPF value and the level of protection provided. Based on this classification, an SPF value of 1-4 provides minimal protection, an SPF value of 4-6 provides moderate protection, an SPF value of 6-8 provides extra protection, an SPF value of 8-15 provides maximum protection, and an SPF value of >15 provides ultra-protection.

Data Analysis

This research is true experimental data obtained from experimental results on the parameters tested. The stability determination result data were processed using the T-test, and the SPF measurement result data were processed using the one-way ANOVA test.

RESULTS AND DISCUSSION

1. Organoleptic and Homogenity Test Result

The organoleptic test results (Figure 1; Table 3) demonstrated that there was a negligible color modification in the spray sunscreen formulations during the 6th cycle. However, the cycle study had no effect on other parameters such as odor, texture and clarity. Stability procedures involving extreme temperatures have a substantial impact on color variations in spray sunscreen preparations over extended periods. High temperature stability and prolonged stability have an impact on the levels of flavonoids as the primary antioxidant agents. It is a known fact that flavonoids are compounds that are sensitive towards alterations in temperature and light (Syafrida *et al.*, 2018). The results of the homogeneity test (Table 3) indicate that the spray sunscreen preparation has reached a good homogeneity, since no insoluble particles were found after the 6th cycle of the test.

2. pH Test Result

The results of pH measurements in the 0th and 6th cycles (Figure 2) obtained a pH of 5.5-6.8. There was a decrease in pH after the cycling test. Statistical analysis obtained significance values in formulas 1, 2, and 3 (p=0.039; p=0.153; p=0.005). The significance value <0.05 (formula 1 and 2) indicated that the cycling test resulted in a significant change in pH. The influence of temperature causes this decrease in pH through the reaction between water and CO2, which can increase the acidity of the preparation (Rabima and Marshall, 2017; Lumentut *et al.*, 2020). However, although statistical changes in pH were found, the final pH value of the preparation was still in a suitable pH range for topical preparations (Salwa *et al.*, 2020). The results of pH measurements explained that all spray sunscreen formulas met the criteria for good physical stability. The suitability of the results of this pH test follows the research of Lumentut *et al.* (2020) regarding the measurement of the pH of the sunscreen which obtained a pH value of 5.06 after a cycle test was carried out for 6 cycles and was declared according to the criteria.

The EE X I value has been set as the reference value and can be seen in Table 2 as follows:

Table 2. Value of EE X I

Wavelength (λ nm)	EE×I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

(Dutra *et al.*, 2004)

Table 3. Organoleptic and homogenity test results at 0^{th} and 6^{th} cycle

Formula	0th Cycle				
	Color	Odor	Texture	Clarity	Homogenity
F1	Green	extract scent	Liquid	Transparent	Homogeneous
F2	Green	extract scent	Liquid	Transparent	Homogeneous
F3	Green	extract scent	Liquid	Transparent	Homogeneous
	6th Cycle				
	Color	Odor	Texture	Clarity	Homogenity
F1	Green	extract scent	Liquid	Transparent	Homogeneous
F2	Green+	extract scent	Liquid	Transparent	Homogeneous
F3	Green++	extract scent	Liquid	Transparent	Homogeneous

Information:
+ : Cloudy green
++ : Murky green

(a) (b)

Figure 1. Organoleptic and homogeneity test results

Information:
(a) Organoleptic and homogeneity test results at 0^{th} cycle
(b) Organoleptic and homogeneity test results at 6^{th} cycle

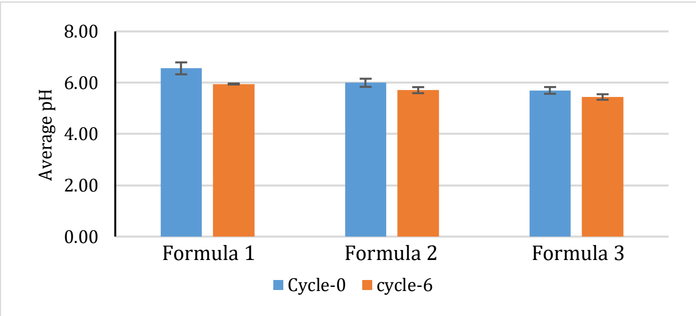


Figure 2. pH value in the 0^{th} and 6^{th} cycles
Information: Formula 1 (500 ppm extract); Formula 2 (1000 ppm extract); Formula 3 (1500 ppm extract).

3. **Viscosity Test**

The results of viscosity measurements in the 0^{th} and 6^{th} cycles (Figure 3) found the viscosity in the range of 1.702-2.737 cPs. There was a decrease in the viscosity value between the 0th and 6th cycles. The temperature influences this decrease in viscosity in the cycling test (Rabima and Marshall, 2017). Statistical analysis results for the viscosity test found that the significance value of formula 1, formula 2, and formula 3 (p=0.307; p=0.109; p=0.171). A significance >0.05 indicates no significant change in the viscosity of the spray sunscreen formulation formulated between the 0th and 6th cycles. Based on the value of the viscosity test results obtained and no significant change found in the viscosity between the 0th and 6th cycles, it can be understood that all of the formulas were physically stable. This result follows good viscosity criteria for spray preparations, with a viscosity range of 500-5000 cPs (Anindhita and Oktaviani, 2020). The results of this research were accorded to the criteria of good and stable physical viscosity based on similar research by Iswandana and Sihombing (2017) on anti-odor spray preparations showed viscosity results in the range of 24,837-30,492 Poise after 6 cycles of cycling test.

4. **Spray Pattern Test**

Measurement of the diameter of the spraying pattern in the 0th and 6th cycles (Figure 4) obtained various diameters. Based on the diameter of the spray pattern, it is known that the decrease affects the change in diameter between the 0th and 6th cycles on the viscosity value. In addition, it was also found that the farther the spraying distance, the wider the diameter formed. These results follow that of Cendana *et al*. (2022), which state that the diameter formed will be directly proportional to the spraying distance.

The results of statistical analysis show varying significance. The significance of formula 1 for distances of 3 cm, 5 cm, 10 cm, and 15 cm is 0.41, 0.11, 0.11, and 0.11 ($p>0.05$). In formula 2 for distances of 3 cm, 5 cm, 10 cm, and 15 cm, respectively, are 0.70, 0.23, 0.04, and 0.07 ($p>0.05$ except at a distance of 10 cm, giving $p< 0.05$). Then in formula 3, 3 cm, 5 cm, 10 cm, and 15 cm are 0.11, 0.11, 0.11, and 0.11, respectively ($p>0.05$). In formula 2, a distance of 10 cm obtained a significance of <0.05, meaning there is a significant difference between the 0th and 6th cycles. Although statistically, there is a significant difference, the diameter of formula 2 at 10 cm between the 0th cycle (14.83 cm) and the 6th cycle (12.92 cm) is similar. The spray pattern diameter measurement showed that all formulas had a stable spray pattern diameter.

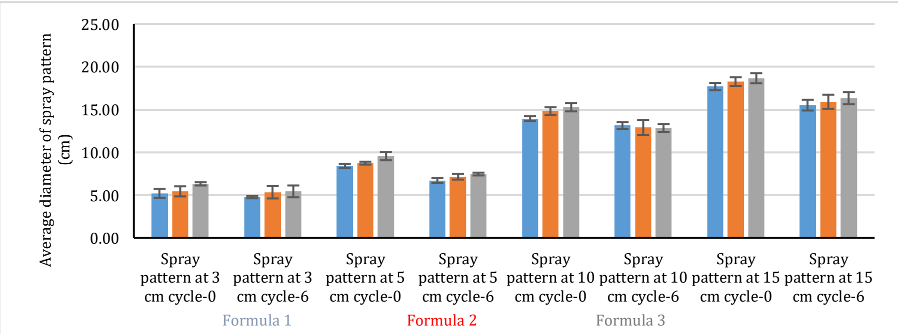
5. **Determination of SPF Value**

The results of the in vitro determination of the SPF value (Figure 5) showed different results. Each formula gave a different SPF value (Formula 1 (18.63); Formula 2 (23.87); Formula 3 (29.56)). SPF value of spray sunscreen preparation when compared to the FDA classification indicates that the sun spray (Formula 1, Formula 2 and Formula 3) provides ultra-protection. The antioxidant effect of the rutin compounds contained in Wungu leaf, which serve as its active ingredients, is responsible for the photoprotective effect of this preparation. These Rutin compounds are part of the Flavonol group of flavonoids. (Singh *et al.*, 2015; Jiangseubchatveera *et al.*, 2017).

Based on the SPF value formed in the positive control formula, it was found that the preparation provided an ultra-protective effect. This photoprotective effect is due to the antioxidant activity of vitamin C, which is used as the active ingredient. The use of vitamin C 1000 ppm as a positive control was based on research by Noviardi *et al*. (2020), which resulted in an SPF value of 14,846. Meanwhile, based on the SPF value formed in the negative control formula, it was found that the preparation provided an extra-protective effect. Although the negative control did not contain the active ingredient, the SPF value was obtained from the content of the chromophore group in the excipients (methylparaben, propylparaben, and BHT) used in the formulation. UV-Vis spectrophotometer will also read this chromophore group and can be relied upon as an SPF value (Suhartati, 2017).

In vitro determination of SPF value shows that spray in each formula provides ultra-protection. The SPF value is sufficient to protect Indonesian skin, which is very diverse. Research by Novitasari *et al*. (2020) found that Indonesian people had III-V skin types. The recommended SPF value for skin types III-V is 15-30 (Juanita and Juliadi, 2020).

Figure 4. Spray pattern diameter in the 0^{th} and 6^{th} cycles



Information: Formula 1 (500 ppm extract); Formula 2 (1000 ppm extract); Formula 3 (1500 ppm extract).

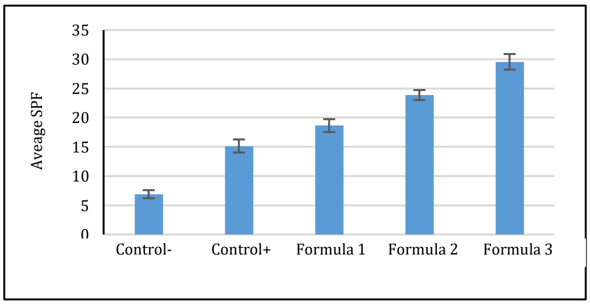


Figure 5. The results of determination SPF value

Information:

Control +	: Positive control (preparation base with vitamin C 1000 ppm)
Control -	: Negative control (preparation base without active ingredients)
Formula 1	: Formula 1 (active ingredient concentration 500 ppm)
Formula 2	: Formula 2 (active ingredient concentration 1000 ppm)
Formula 3	: Formula 3 (active ingredient concentration 1500 ppm)
*	: Significantly different to Control+ (P=0.014) and Control- (P=0.010)
**	: Significantly different to Control+ (P=0.014)

Statistical analysis of the SPF value demonstrated a significance of < 0.05 (p=0.009) , indicating a significant association between extract concentration and SPF value (Figure 5). The results show that as extract concentration increases, SPF value also increases. This finding is consistent with research by Lisnawati *et al.* (2019), which found that higher extract concentrations lead to higher SPF values.

CONCLUSIONS

This study shows that the formulation of a spray sunscreen with Wungu leaf extract provides an ultra-protective level of photoprotection, as evidenced by the SPF values produced by Formula 1 (18.63), Formula 2 (23.87) and Formula 3 (29.56). In addition, the sunscreen has good physical stability based on the parameters measured, including organoleptic (color, odor, texture and clarity), homogeneity, pH, viscosity and spray pattern diameter.

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CONFLICT OF INTEREST

All authors declared that there was no conflict of interest.

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