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Formulation and Stability of Miana Leaf Extract Spray Gel with Antioxidant

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ABSTRACT. The extract of Miana leaves (*Coleus scutellarioides* L.) is recognized for possessing potent antioxidant Action. This research aimed to develop a physically stable spray gel incorporating miana leaf extract and to evaluate its antioxidant potential through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method. Three formulations with different proportions of Carbopol and hydroxypropyl methylcellulose (HPMC) were evaluated for stability under room-temperature storage and during cycling tests, with organoleptic characteristics and viscosity as assessment parameters. The extract alone exhibited very strong antioxidant Action (IC_{50} = 47.71 ppm). In comparison, the spray gel formulations showed IC_{50} values of 141.18 ppm (FE1, 1%), 113.72 ppm (FE2, 3%), and 80.90 ppm (FE3, 5%), corresponding to moderate, moderate, and strong Action, respectively. Among these, FE2 (3% extract) demonstrated the most favorable physical stability.

INTRODUCTION

The skin is the outermost organ and is important as a physical barrier against external influences. Exposure to free radicals, such as ultraviolet (UV) rays, can cause skin damage (Purnawaningsih *et al*., 2014). Excessive exposure to free radicals may lead to adverse skin effects, including premature aging, skin cancer, and reduced immune function (Jain and Jain, 2010). One way to counter the harmful effects of free radicals is to use products containing antioxidants. Antioxidants are bioactive compounds that protect the body by neutralizing free radicals. Apart from that, antioxidants can also provide many benefits for skin health, such as promoting skin regeneration and reducing wrinkles caused by premature aging (Arisanti and Mutsyahidan, 2018).

Nowadays, the back-to-nature trend in the use of skin care products in Indonesian society is growing rapidly. Formulating cosmetic preparations from natural ingredients can provide greater comfort in use, trust, and societal acceptance, thereby increasing the number of cosmetic products containing plant extracts (Chen, 2011; Indonesian Herbal Pharmacopoeia, 2017). Phenolic or polyphenolic compounds, such as flavonoids, can act as natural antioxidants found in many plants. Flavonoid compounds acted as antioxidants because they have hydroxyl groups that can release protons as hydrogen ions. The high flavonoid and phenol content will act as antioxidants, helping fight free radicals (Liew *et al*., 2018).

Traditional medicine generally uses various plants known to possess pharmacological activity. Indonesia, one of the world's countries with the greatest biodiversity, provides an abundant botanical resource that can be developed into therapeutic agents. One example of a plant with potential medicinal value is the Miana (Hilma *et al*., 2020). Miana (*Coleus scutellarioides* L.) is a plant rich in flavonoids and belongs to the Lamiaceae family, widely cultivated in Indonesia and often used to treat various diseases (Salimi, 2021). Research results from Anita *et al*. (2018) show that the ethanol extract of Miana leaves (*C. scutellarioides* L.) contains polar compounds, such as flavonoids, tannins, alkaloids, and saponins. Sari (2013) reported that miana leaves (*C. atropurpureus* L.) contain flavonoids with considerable antioxidant potential, indicated by an IC_{50} value of 48.04 ppm in the ethanol extract. Khotimah *et al*. (2018) reported that the ethanol extract of Miana leaves had strong antioxidant activity

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(IC_{50} 70.13 ppm) and that during cool-temperature storage, antioxidant activity became more stable and increased (Khotimah *et al*., 2018). Hardiyanti *et al*. (2013) also reported that miana leaf extract contains antioxidant substances, such as anthocyanins, and has an antioxidant activity of 84.64% (Hardiyanti *et al*., 2013). The types of anthocyanins found in Miana (*C. scutellarioides*) leaves are pelargonidin, cyanidin, peonidin, and delphinidin (Lestario *et al*., 2009). Rusli *et al*. (2019) formulated a Miana leaf ethanol extract at 2.5% concentration into a sunscreen lotion, which received an extra protection category. Furthermore, Ulfah *et al*. (2016) demonstrated that the antioxidant Action of Miana (*Coleus scutellarioides* L.) leaf extract gel preparations at a concentration of 1% was stable at room temperature and freeze-thaw storage.

Given the antioxidant activity of Miana leaves, it is very interesting to develop an innovative pharmaceutical preparation. Spray gel is one of the pharmaceutical preparations that can make its use easier. The advantage of spray gel preparations is that they have a low level of microbial contamination because they can be delivered directly to the skin without contact with the hands, making them easier and more practical to use. Apart from that, spray gel preparations can also provide a pleasant experience for users (Salwa *et al*., 2020). Thus, researchers are interested in examining miana leaves formulated into a spray gel with antioxidant activity.

A gelling agent is an important factor in formulating a high-quality spray gel that meets the requirements (Fissy *et al*., 2013). The final quality and physical properties of spray gel preparations are significantly influenced by the gelling agents used. These include organoleptics, pH, viscosity, homogeneity, spray power, and stability, which need to be considered to obtain a good preparation that is easy to apply, comfortable, and that meets the requirements (Kresnawati *et al*., 2022). The combination of hydroxypropyl methylcellulose (HPMC) and carbopol gelling agents can provide a good and stable consistency because the crosslink between the two polymers is stronger (Rowe *et al*., 2017). Therefore, this study was conducted to develop a spray gel formulation of miana leaf extract and to assess its physical stability and antioxidant activity at extract proportions of 1%, 3%, and 5%

RESEARCH METHODS

Miana leaf samples were collected from TP Sungkai Farm, Bogor, West Java, between November 2022 and January 2023, and were taxonomically identified at the National Research and Innovation Agency (BRIN) as *Coleus scutellarioides* L. Benth. The other materials used were ethanol 96%, ethanol p.a, 1,1-diphenyl-2-picrylhydrazyl (DPPH), beeswax, and vaseline flavum. The instruments used were a set of vacuum rotary evaporators (Eyela), a refrigerator (Sanyo Medicool), and a UV-Vis spectrophotometer (Hitachi U-2910).

Miana Leaf Extraction

Determination of Specific Parameters of Miana Leaf Extract

Organoleptic tests were performed by observing the extract's appearance, coloration, and characteristic odor. Phytochemical profiling was conducted using qualitative tube assays to identify the presence of alkaloids, flavonoids, tannins, terpenoids and steroids, saponins, and phenolic compounds (Utami *et al*., 2017).

Determination of Non-Specific Parameters of Miana Leaf Extract

The extract water content is determined using the gravimetric method. The determination of extract ash content was carried out according to the method described in the Indonesian Herbal Pharmacopoeia (2017).

Determination of Non-Specific Parameters of Miana Leaf Extract using the DPPH Method

Preparation of 0.05 mM DPPH Stock Resolution

DPPH resolution was prepared by dissolving 2 mg of 2,2-diphenyl-1-picrylhydrazyl in a 100 mL volumetric flask with ethanol p.a, followed by mixing until homogeneous (Hasanah *et al*., 2017).

Determination of DPPH Maximum Wavelength

The absorbance of a mixture of 2 mL 0.05 mM DPPH stock solution and 1 mL ethanol p.a. was measured after 30 minutes of incubation in the dark using a UV-Vis spectrophotometer at 400 – 600 nm (Hasanah *et al*., 2017; Martiani *et al*., 2017).

Preparation and Measurement of Vitamin C Comparator Resolutions

Vitamin C powder weighing 5 mg was placed into a 50 mL volumetric flask, ethanol p.a. was added to the mark line, and the mixture was shaken until homogeneous to obtain a 100 ppm concentration of vitamin C solution. Next, a series of vitamin C solutions at 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm was prepared for comparison testing. One milliliter of each test solution series was pipetted into a brown vial, then 2 mL of 0.05 mM DPPH solution was added, and the mixture was shaken until homogeneous. All test solutions were incubated in the dark

for 30 minutes, protected from light, and their absorbance was measured at the maximum wavelength using a UV-Vis spectrophotometer (Martiani *et al*., 2017).

Preparation and Measurement of Extract Test Resolutions

An extract weighing 50 mg was placed into a 50 mL volumetric flask, then ethanol p.a. was added to the mark line, and the mixture was shaken until homogeneous to obtain a 1000 ppm concentration. Next, a concentration series of test resolutions was prepared at 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. One milliliter of each solution was pipetted into a brown vial, then 2 mL of 0.05 mM DPPH solution was added, and the mixture was shaken until homogeneous. All test solutions were incubated in the dark for 30 minutes, protected from light, and their absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength (Martiani *et al*., 2017).

Determination of IC_{50}

The percentage value of inhibition of DPPH radicals from each concentration of the test solution can be calculated using Equation 1.

$$\%\ \text{inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \quad (1)$$

The percent inhibition results for each concentration are plotted as a linear curve, and the equation y = bx + a is obtained, with the x-axis representing the test concentration (ppm) and the y-axis representing the percent inhibition. The concentration of the test sample that reduces DPPH radicals by 50% is called the antioxidant activity and is expressed as IC_{50} (Inhibition Concentration 50%). The x-value is the IC50 when the y-value is set to 50 (Angelia *et al*., 2022).

Determination of AAI Value

The Antioxidant Activity Index (AAI) value can be determined using Equation 2.

$$\text{AAI Value} = \frac{\text{DPPH concentration used in the test (ppm)}}{IC_{50}\ \text{value obtained (ppm)}} \quad (2)$$

Optimization of Spray Base Gel

Carbopol was dispersed by sprinkling 20 mL onto distilled water, then homogenized quickly by stirring in a mortar until completely dispersed. Afterward, triethanolamine (TEA) was added and mixed until a transparent gel mass (M1) formed. HPMC was dispersed in 20× the amount of HPMC in hot distilled water, then mixed until a gel mass (M2) formed. Then M1 and M2 were stirred using an overhead stirrer until a clear gel mass (M3) formed. Menthol was dissolved in ethanol until completely dissolved and added to the M3 gel mass, then mixed until homogeneous. Propyl paraben and methyl paraben were dissolved in propylene glycol, stirred until evenly mixed, then added to the M3 mixture and stirred until homogeneous. After all the mixtures were homogeneous, the remaining distilled water was added, and the mixture was weighed to reach the specified weight.

Table 1. Spray base gel formula with modifications (Ramdha and Azizah, 2021).

Ingredient	Function	Concentration (%)		
		FB1	FB2	FB3
Carbopol 940	Gelling agent	0.4	0.6	0.4
HPMC	Gelling agent	0.4	0.4	0.6
Propylene glycol	Humectan	5	5	5
TEA	Alkalizing agent	0.25	0.25	0.25
Methyl paraben	Antimicrobial preservative	0.18	0.18	0.18
Propylparaben	Antimicrobial preservative	0.02	0.02	0.02
Menthol	Enhancer	0.05	0.05	0.05
Etanol 95%	Solvent	5	5	5
Aquadest ad	Solvent	100	100	100

FB1 = Carbopol:HPMC = 0.4%:0.4%
FB2 = Carbopol:HPMC = 0.6%:0.4%
FB3 = Carbopol:HPMC = 0.4%:0.6%

Miana Leaf Extract Gel Spray

The spray gel formulations with different concentrations of Miana leaf extract are presented in Table 2.

Table 2. Miana leaf extract spray gel formula.

Ingredient	Function	Concentration (%)		
		FE1	FE2	FE3
Miana Leaf Extract	Active ingredient	1%	3%	5%
Carbopol 940	Gelling agent	According to the best formula concentration		
HPMC	Gelling agent			
Propylene Glycol	Humectant	5	5	5
TEA	Alkalizing agent	0.25	0.25	0.25
Methyl paraben	Antimicrobial preservative	0.18	0.18	0.18
Propyl paraben	Antimicrobial preservative	0.02	0.02	0.02
Menthol	Enhancer	0.05	0.05	0.05
Ethanol 95%	Solvent	5	5	5
Aquadest	Solvent	100	100	100

FE1 = Miana Leaf Extract Spray Gel 1%
FE2 = Miana Leaf Extract Spray Gel 3%
FE3 = Miana Leaf Extract Spray Gel 5%

Manufacturing Procedure

The spray base gel that was made was supplemented with Miana leaf extract at concentrations of 1%, 3%, and 5%. Then stir until the base and the extract are fully combined.

Physical Assessment of The Prepared Spray Gel

Organoleptic

The physical appearance of the spray gel was visually observed, including odor, color, and texture (Angelia *et al*., 2022).

Homogeneity

A small amount of the prepared spray gel was applied to a glass slide, and the resulting phenomenon was observed. The prepared gel is considered homogeneous if no coarse grains are observed (Angelia *et al*., 2022).

Viscosity

A sample of 100 mL from the prepared spray gel was analyzed for viscosity using a Brookfield LV viscometer fitted with spindle no 64 at a rotation speed of 30 rpm. The prepared spray gel has a good viscosity in the range of 500–5000 cPs (Angelia *et al*., 2022).

pH

The pH of the formulation was determined using a calibrated pH meter. One gram of the prepared spray gel was dissolved in 10 mL of distilled water. The preparation must fall within the pH range of normal human skin, which is around 4.5 – 6.5 (Liony, 2014).

Spray Pattern

In this test, the spray gel was sprayed onto plastic sheets that had been previously weighed and labeled, at distances of 3 cm, 5 cm, 10 cm, and 15 cm. The test was carried out 3 times, and the spray formation pattern, spray pattern diameter, and weight per spray were observed (Suyudi, 2014).

Sticky Spreadability

The spray gel was applied once to the clean, dry skin of the upper arm from a distance of 3 cm, followed by a 10-second count using a stopwatch. The test was conducted three times, and observations were made on the spray pattern, its diameter, and the weight per spray (Suyudi, 2014).

Cycling Test

The cycling test for physical stability existed conducted over 6 cycles, where in each cycle the spray gel preparation existed placed in the refrigerator at a cold temperature (4 ± 2 °C) for 24 hours and continued by placing the spray gel preparation in the oven at a hot temperature (40 ± 2 °C) for 24 hours.

Antioxidant Activity Test of Spray Gel Preparations

A 25 mg sample of spray gel of Miana lead extract was then dissolved in ethanol p.a. in a 25 mL volumetric flask to obtain a concentration of 1000 ppm. Miana leaf extract solutions were prepared in series at concentrations

of 5, 50, 100, 200, and 300 ppm. One milliliter of each concentration was transferred to a brown vial, 2 mL of DPPH stock solution was added, and the mixture was incubated for 30 minutes in the dark, protected from light. The absorbance was then measured using a UV-Vis spectrophotometer at the maximum wavelength (Martiani *et al*., 2017). The IC_{50} and AAI values were determined by following the steps in the antioxidant activity test procedure for Miana leaf extract.

RESULTS AND DISCUSSION

Miana Leaf Extraction

An amount of 3.279 g of simplicia powder was extracted through the maceration technique using 96% ethanol as the extraction solvent. The concentrated extract of Miana leaves was obtained at 329 g, yielding 11.96%. This complies with the requirement that the yield of concentrated Miana leaf extract should not be less than 9% (Indonesian Herbal Pharmacopoeia, 2017). Organoleptically, miana leaf extract is a thick mass of dark purple-brown color and has a distinctive odor. Phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, steroids, saponins, and phenolic compounds in the miana leaf extract. According to the Indonesian Herbal Pharmacopoeia (2017), the required extract water content is generally less than 10%. Based on the test results, the water content of Miana leaf extract is 9.992%, indicating that it meets the water content requirements. The ash content of Miana leaf extract was 3.670%. The higher the ash content indicates the higher the mineral content in the extract.

Miana Leaf Extract Antioxidant Activity Test

Testing of Miana leaf antioxidant activity was performed through the DPPH method. The DPPH method was selected for its simplicity, rapidity, and sensitivity, while requiring only a small sample volume. The results of the DPPH test can be seen by observing the change in color of the test sample from purple to yellow, which indicates that DPPH has been reduced by the process of donating hydrogen or electrons from antioxidant compounds, so that the color changes to yellow and does not provide absorption at a wavelength of 517 nm. Based on the test results, the maximum DPPH absorbance was observed at 517 nm. This wavelength is used to measure the test sample.

Table 3. Vitamin C antioxidant activity test results.

Concentration (ppm)	Average absorbance	% inhibition	IC_{50} (ppm)	AAI
10	0.275	66.854		
8	0.373	55.056		
6	0.472	43.218	7.17	2.79
4	0.579	30.337		
2	0.664	20.024		

In this study, absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. The IC_{50} value for vitamin C was 7.17 ppm, placing it in the very strong category. Vitamin C was used as a comparison (positive control) because Vitamin C has very high antioxidant Action (Lung and Destiani, 2017).

Table 4. Results of the Miana leaf extract antioxidant activity test.

Concentration (ppm)	Average absorbance	% inhibition	IC_{50} (ppm)	AAI
100	0.148	82.143		
80	0.261	68.579		
60	0.356	57.103	47.71	0.42
40	0.474	42.937		
20	0.534	35.674		

In this study, absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength. The IC_{50} value for miana leaf extract is found to be 47.71 ppm, which falls into the strong category. When compared with Vitamin C, which had an IC_{50} value of 7.17 ppm and is classified as very strong, the antioxidant activity of Miana leaf extract corresponded to 15.01% of that of Vitamin C. The lower antioxidant activity of the extract than Vitamin C could be because Vitamin C is a very pure compound, while Miana leaf extract is a crude extract, which still contains many secondary metabolites, not pure compounds or isolates. Compounds in Miana leaf extract

suspected to have antioxidant activity include flavonoids and phenolic compounds, which can prevent free radicals by donating hydrogen atoms to radical species (Amin *et al*., 2016).

Physical Assessment of Spray Base Gel

Organoleptic Test

The spray gel prepared by the three formulas is clear or transparent and has a distinctive menthol odor. The dosage forms of FB1, FB2, and FB3 are in the form of slightly thick, thick, and very thick gel liquids, respectively. The three base formulas produce gels that are organoleptically stable when stored at room temperature from days 0, 7, 14, and 21. This is because the base preparations do not experience changes in color, odor, shape, or mold growth. FB2 has the most bubbles compared to FB1 and FB3. This is because the concentration of carbopol used in FB2 is higher compared to FB1 and FB2. Air bubbles formed in the gel preparation after carbopol was neutralized with base. This can occur when a base is added to carbopol immediately after it is dispersed in water, resulting in the gel trapping air and forming bubbles (Suyudi, 2014). The higher the concentration of carbopol, the more bubbles will form (Hidayanti *et al*., 2015). Meanwhile, the longer the storage period, the fewer trapped air bubbles will be (Sihombing and Lestari, 2015). This is shown in the three formulas, where the number of air bubbles decreases from day 0 to day 21.

Homogeneity Test

The three base formulas depict a homogeneous, evenly distributed mixture of particles. This is because the third formula shows no particles or coarse grains that are not homogeneous; all particles are evenly distributed, and no lumpy gel forms.

pH Test

The base-optimized spray gel prepared has a pH range of 5.85 – 6.17, indicating that each formula meets the pH requirements for topical preparations (Table 5). Compatibility of this pH range with the skin is very important for safety, as it is more easily accepted by the skin and does not cause pain, irritation, or injury (Aprilianti *et al*., 2020). There are differences in the pH values of the formulas, which are due to the concentration of the gelling agent used. An increase in carbopol concentration results in a decrease in pH value.

Table 5. Results of pH optimization testing for spray base gel.

Day-	pH value		
	FB1	FB2	FB3
0	5.97 ± 0.015	5.85 ± 0.020	6.11 ± 0.020
7	6.00 ± 0.035	5.90 ± 0.026	6.12 ± 0.021
14	6.02 ± 0.021	5.98 ± 0.031*	6.14 ± 0.031
21	6.04 ± 0.026	5.98 ± 0.046*	6.17 ± 0.036

*different meaning p (<0.05)

Viscosity Test

Spray gels prepared with a viscosity of 500–5000 cPs produce a good preparation (Kamishita *et al*., 1992). The three formulas have viscosity values within the range required for spray gel preparations and indicate preparations with a thick consistency. The differences in viscosity among the three formulas are due to the different proportions of the gelling agent used. The higher the concentration of the gelling agent, the higher the viscosity (Table 6).

Table 6. The base optimization viscosity test results.

Day-	Viscosity Value (cPs)		
	FB1	FB2	FB3
0	2100 ± 100	3400 ± 100	2533 ± 58
7	2200 ± 100	3467 ± 153	2833 ± 208
14	2223 ± 153	3500 ± 100	3033 ± 153*
21	2400 ± 100	3667 ± 153	3100 ± 100*

*different meaning p (<0,05)

Spray Pattern Test

The test results for spray patterns FB1, FB2, and FB3 vary as shown in Table 7. The spray distance is directly proportional to the spray pattern diameter; greater distances produce larger diameters. The spray patterns in

formulas FB1, FB2, and FB3 are confined to a single straight line from the spray, forming a small circle with an average diameter of 1.7 – 3.0 cm. The results of measuring the weight of the one-time spray show that the base-optimized spray gel prepared has a uniform weight per spray.

Table 7. Diameter per spray of base optimization test results.

Formula	Spraying Distance (cm)			
	3	5	10	15
FB1	2.5 ± 0.058*	2.5 ± 0.153*	2.8 ± 0.265	3.0 ± 0.252
FB2	2.1 ± 0.100	2.5 ± 0.058	2.2 ± 0.321	2.3 ± 0.153
FB3	1.7 ± 0.208	1.7 ± 0.100	2.0 ± 0.153	2.1 ± 0.200

* different meaning p (<0,05)

Sticky Spreadability Test

A good spray gel must show that the prepared gel adheres to the upper arm skin after spraying and remains attached for more than 10 seconds. Based on the test results, FB1, FB2, and FB3 show preparations that can adhere to the upper arm skin for more than 10 seconds after being sprayed. The three formulas show a lack of spreadability: they do not spread evenly when sprayed and do not just accumulate at a single spray point, so they are not suitable for meeting the requirements for a good spray gel. This can be caused by the preparation's high viscosity, which leads to larger particle sizes. Apart from that, the valve on the applicator and flow characteristics can also affect the spraying results. Thixotropic flow properties are advantageous for topical preparations because they provide high consistency in the container but are easy to spread, making application easier (Chandra and Fitria, 2019).

Cycling Test

The spray base gel optimization preparation showed no change in color, odor, or shape between before and after the cycling test. This shows that the optimized spray base gel preparation is homogeneous and evenly dispersed. The bubbles in the preparation decreased after the cycling test. The pH test results showed that the pH of the preparations after the cycling test did not change significantly, with all formulas maintaining pH values within the skin's pH range of 4.5 – 6.5. In the viscosity test, the preparation's viscosity increased after the cycling test. An increase in viscosity can occur along with an increase in the pH of the preparation. This is because the viscosity of carbopol increases with increasing pH and decreases at pH values below 3 and above 12. This increase in consistency can be caused by the evaporation of one of the solvent components in the formula, which is ethanol (Praptiwi *et al*., 2014). Of the three formulas, only FB3 shows a viscosity shift that did not meet the requirements, with a 16.67% of viscosity shift value. The stability of the preparation can be seen from the shift in viscosity during storage. A prepared spray gel is considered to have good stability if its viscosity shift is less than 10% (Nurdianti, 2018). All three formulations adhered to the upper arm skin for over 10 seconds, remaining localized at the point of application. Spray diameter increased with spraying distance, while the weight per spray showed no significant change before and after the cycling test.

Physical Assessment of Miana Leaf Extract Gel Spray Preparation

The formulation of the spray gel preparation for Miana leaf extract was carried out after determining the optimal formula from the base optimization results. The formula for the Miana leaf extract spray gel is the same as the formula used for carbopol:HPMC, with a ratio of 0.4%:0.4 %, TEA, propylene glycol, methyl paraben, propyl paraben, menthol, and distilled water. Miana leaf extract was added to this formula at 3 concentrations, resulting in 3 formulas: FE1 at 1%, FE2 at 3%, and FE3 at 5%.

Organoleptic Test

Organoleptically, the three Miana leaf extract spray gel formulas have a slightly thick gel texture, a distinctive odor, and a brownish color with varying levels of intensity. This is because the extract concentration differs across formulas; the higher the concentration, the more intense the preparation's color. No changes in the shape, color, or odor of the three formulas occurred on days 0, 7, 14, and 21. Thus, it can be concluded that the spray gel preparation of Miana leaf extract is stable at room temperature storage.

Homogeneity Test

Visually, the test results for the three formulas show that the preparations are less homogeneous, as small particles originating from the Miana leaf extract are present. However, there are no lumps in the base gel, indicating that it is completely dispersed. The higher the concentration of the extract, the more particles there will be in the

preparation. The presence of these particles can be caused by environmental influences such as temperature, light, air, and humidity (Gustavina *et al*., 2017).

pH Test

The three spray gel formulas show that the prepared spray gel has a good pH value and meets the requirements, which are 4.5 – 6.5 (Table 8). The differences in pH between FE1, FE2, and FE3 are due to the presence of flavonoid compounds in the Miana leaf extract, which are slightly acidic (Podungge *et al*., 2017). The higher the extract concentration, the lower the pH of the preparation will be.

Table 8. pH test results for Miana leaf extract spray gel.

Day-	pH Value		
	FE1	FE2	FE3
0	6.12 ± 0.026	5.88 ± 0.030	5.69 ± 0.032
7	6.13 ± 0.025	5.90 ± 0.030	5.72 ± 0.031
14	6.16 ± 0.021	5.92 ± 0.031	5.77 ± 0.025*
21	6.18 ± 0.031	5.93 ± 0.020	5.82 ± 0.036*

* different meaning p (<0,05)

Viscosity Test

The three Miana leaf extract spray gel formulas have viscosities that meet the requirements. FE3 has a lower viscosity compared to FE1 and FE2 (Table 9). This can be caused by the acidic nature of miana leaf extract, which results in repulsion between carboxyl groups, breaking the carbopol polymer chain, and reducing the viscosity of the gel (Ansiah, 2014). Thus, the higher the concentration of Miana leaf extract used, the lower the viscosity of the preparation will be.

Table 9. Viscosity test results for Miana leaf extract spray gel.

Day-	Viscosity Value (cPs)		
	FE1	FE2	FE3
0	2333 ±153	2200 ± 100	2033 ± 153
7	2367 ± 58	2300 ± 100	2200 ± 100
14	2533 ± 58	2367 ± 153	2300 ± 100
21	2633 ± 153*	2500 ± 153	2367 ± 153

* different meaning p (<0,05)

Spray Pattern Test

The spraying patterns of FE1, FE2, and FE3 formed small circular areas with average diameters of 1.4 – 1.9 cm (Table 10). The spray diameter increased with greater spraying distance, while the weight per spray remained consistent across all formulations.

Table 10. Diameter test results per spray of Miana leaf extract spray gel.

Formula	Spraying Distance (cm)			
	3	5	10	15
FE1	1.5 ± 0.058	1.5 ± 0.100	1.7 ± 0.208	1.8 ± 0.208
FE2	1.4 ± 0.100	15 ± 0.058	1.5 ± 0.115	1.6 ± 0.100
FE3	1.6 ± 0.100	1.8 ± 0.115	1.9 ± 0.173	1.9 ± 0.153

* significantly different at $p < 0.05$

Sticky Spreadability Test

Based on test results, the three formulas can adhere to the upper arm skin for more than 10 seconds after being sprayed. However, the three formulas showed that the dispersion power did not spread evenly during spraying and did not accumulate at a single spray point, so they were not suitable for meeting the requirements for a good spray gel product.

Cycling Test

The results of the organoleptic test for the spray gel of Miana leaf extract showed no change in color, odor, or shape before and after the cycling test. This shows that the spray-gel preparation of Miana leaf extract is evenly dispersed. The Miana leaf extract spray gel preparation did not show any bubbles. There are small particles in the Miana leaf extract spray gel derived from Miana leaf extract. The higher the extract concentration, the more extract

particles there will be in the preparation. The pH test results show that the product's pH after the cycling test has increased but remains within the skin pH range of 4.5 – 6.5.

In the viscosity test, the preparation's viscosity increased after the cycling test, but remained within the range required for a good spray gel (500 – 5000 cPs). Among the three formulas, only FE2 shows a viscosity shift that meets the requirements, with a shift value of less than 10%. In the adhesive spreadability test before and after the cycling test, the three formulas showed that the preparation could stick after being sprayed on the upper arm skin and could remain for more than 10 seconds, but did not spread and only accumulated at a single spray point. In the spray pattern test, the diameters of the three formulas are directly proportional to the spraying distance; the farther the spraying distance, the larger the spray diameter. The results of measuring the weight per spray for each formula also did not show a significant difference between the tests before and after the cycling test, indicating that the preparations had a uniform weight.

Founded on the results of the physical Assessment of the Miana leaf extract spray gel preparation, the most physically stable Miana leaf extract spray gel formula was obtained, namely FE2, with a Miana leaf extract concentration of 3%.

Antioxidant Activity Test of Miana Leaf Extract Spray Gel

The results of antioxidant testing for the Miana leaf extract spray gel are listed in Table 11. Based on the IC50 value, the antioxidant activities of FE1, FE2, and FE3 are medium, medium, and strong, respectively, with the AAI being weak across the three formulas. This can be caused by the higher concentration used in FE3 compared to FE1 and FE2. The findings indicate that increasing the concentration of Miana leaf extract decreases the IC50 value, indicating an enhancement in antioxidant Activity against free radicals. The antioxidant Action of the Miana leaf extract spray gel preparation may also be influenced by other components, namely menthol. Menthol can act as an antioxidant because it contains a phenol group, which has the ability to neutralize free radicals (Gökalp, 2015).

Table 11. Antioxidant activity test results of Miana leaf extract spray gel.

Test Sample	Concentration ppm)	Average absorbance	% Inhibition	IC_{50} (ppm)	AAI
F0	5	0.735	10	371.21	0.05
	50	0.683	17		
	100	0.666	19		
	200	0.558	32		
	300	0.471	42		
FE1	5	0.513	37	141.18	0.14
	50	0.473	42		
	100	0.433	47		
	200	0.362	56		
	300	0.303	63		
FE2	5	0.504	38	113.72	0.18
	50	0.455	44		
	100	0.415	49		
	200	0.333	59		
	300	0.272	67		
FE3	5	0.484	41	80.90	0.25
	50	0.429	48		
	100	0.385	53		
	200	0.318	61		
	300	0.208	75		

F0 = spray Gel without Miana Leaf Extract

The antioxidant activity between the base and the Miana-derived spray gel is significantly different, indicating that the addition of the extract significantly influences the spray gel's antioxidant activity, although the base alone can provide antioxidant activity. In addition, the concentration of menthol in the base and the Miana-derived spray gel is very low, so it has little effect on antioxidant activity. The preparation process can also affect antioxidant activity. This is because flavonoids and antioxidant compounds are sensitive to temperature and light

(Zainol *et al*., 2009). Founded on the results of comparing the IC_{50} value of the Miana leaf extract spray gel preparation against Miana leaf extract, the antioxidant activity of the Miana leaf extract spray gel preparation on FE1, FE2, and FE3 existed respectively 33.79%, 41.96%, and 58.98%. The higher the percentage, the greater the antioxidant activity in the spray gel preparation.

CONCLUSION

The Miana leaf extract demonstrated very strong antioxidant activity, with an IC_{50} value of 47.71 ppm. The optimized spray-gel base that showed good physical stability was formula FB1 (carbopol:HPMC = 0.4%:0.4%). Meanwhile, the spray-gel formulation containing Miana leaf extract that remained stable was FE2, which used a 3% extract concentration. Antioxidant activity testing indicated that FE1 (IC_{50} = 141.18 ppm) and FE2 (IC_{50} = 113.72 ppm) fell into the moderate category, whereas FE3 (IC_{50} = 80.90 ppm) was categorized as strong.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

AUTHOR CONTRIBUTION

NS: Conceptualization, Supervision, Project Administration, Resources; RA: Methodology, Data Analysis, Supervision, Software; VA: Methodology, Validation, Data Analysis, Supervision; TS: Manuscript Drafting, Data Analysis, Conceptualization, Methodology; SD: Manuscript Review, Investigation, Validation; OSB: Manuscript Review, Investigation; EMD: Methodology, Validation, Data Analysis.

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