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Formulation and Determination of Spf (*Sun Protection Factor*) Mulberry *Leaf Extract Spray Gel Preparation (Morus* Nigra *L.)* As Sunscreen in Vitro

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ABSTRACT

Excessive exposure to ultraviolet (UV) radiation is associated with a wide range of adverse skin effects, including erythema, photoageing, and an increased risk of skin cancer, highlighting the need for effective photoprotective formulations. This study aimed to formulate a spray gel containing mulberry leaf (*Morus nigra* L.) extract and to evaluate its *in vitro* Sun Protection Factor (SPF) alongside its physicochemical characteristics. Mulberry leaf extract was incorporated into spray gel formulations at concentrations of 0.75% (F1), 3.75% (F2), and 6.75% (F3). All formulations were prepared in triplicate and evaluated for organoleptic properties, homogeneity, pH, viscosity, spray pattern, and *in vitro* SPF using the UV–Visible spectrophotometric method based on the Mansur equation. Statistical analysis was performed using the Shapiro–Wilk and Levene tests followed by appropriate non-parametric analysis when assumptions of normality and homogeneity were not satisfied ($p < 0.05$). The mean SPF values obtained were 25.09 (Ultra), 25.74 (Ultra), and 36.98 (Ultra) for F1, F2, and F3, respectively, demonstrating a concentration-dependent increase in photoprotective activity. All formulations exhibited acceptable physicochemical characteristics for spray gel preparations throughout the evaluation period. Among the tested formulations, F3 (6.75%) produced the highest *in vitro* SPF value. These findings indicate that *Morus nigra* leaf extract possesses promising *in vitro* photoprotective potential for spray gel formulations. However, further stability, photostability, skin compatibility, and *in vivo* studies are required before practical application can be recommended.

INTRODUCTION

Indonesia, as a tropical country located along the equator, experiences the highest intensity of ultraviolet (UV) radiation, which typically decreases with increasing latitude. Ultraviolet (UV) radiation is a solar spectrum that carries the highest potential for skin aging and skin cancer (Azzahra et al., 2023). As the body's largest organ, the skin functions to protect the body against UV radiation exposure. Melanocytes in the skin produce melanin, which plays a major role in forming eumelanin as a primary protective factor against UV exposure (280–400 nm), as brown/black pigments possess photoprotective properties. Melanin absorbs radiation energy and dissipates it as heat, thereby suppressing DNA damage (Putranti & Sistina, 2023).

Long-term exposure to UV radiation can lead to a decline in the body's immune system, which is associated with malignancy and photoaging (Putranti & Sistina, 2023). According to (Dampati & Veronica, 2020), the annual incidence of skin cancer in Indonesia is approximately 5.9% to 7.8%. Furthermore, prolonged UV exposure also has the potential to cause sunburn, hyperpigmentation, erythema, and skin darkening (Dampati & Veronica, 2020).

The harmful effects associated with prolonged UV exposure may be reduced through the regular use of sunscreen preparations. Synthetic sunscreen products are often associated with a greater risk of skin irritation, which has increased interest in naturally derived alternatives that are generally considered safer and more skin-friendly, especially for hyper-allergic skin. Natural raw materials are also relatively affordable and easy to obtain (Meilina et al., 2023). Topical sunscreens have been developed in various forms, one of which is the spray gel. The spray gel dosage form is applied via spray without direct hand contact, which

prevents microbial contamination and offers greater efficiency and ease of use compared to other topical forms (Kresnawati et al., 2022). Additionally, spray gels provide a longer penetration time for active ingredients, dry quickly, and produce a cooling effect upon application (Pebriani et al., 2023). Natural ingredients with potential as sunscreens generally contain antioxidant compounds (Dampati & Veronica, 2020).

The Mulberry plant (*Morus nigra* L.) is well-known as a source of antioxidant compounds capable of neutralizing free radicals and playing a vital role in healing degenerative diseases (Salampe et al., 2025). Research by (Purnama, 2022) indicates that compared to the fruit, mulberry leaves contain higher levels of total phenols and flavonoids. Mulberry leaves contain several active compounds, including alkaloids, flavonoids, polyphenols, and terpenoids, all of which act as antioxidants (Pogaga et al., 2020). A study by (Reinard et al. 2022) demonstrated that a gel preparation with a 9% concentration of mulberry leaf extract yielded an IC (50) value of 52.122 ppm, indicating strong antioxidant activity and sunscreen potential. This is further supported by the research of (Tania et al., 2025) which analyzed secondary metabolite profiles and SPF (Sun Protection Factor) values; their results proved that mulberry leaves have sunscreen potential, with an average SPF of 3.19 (minimal category) at 300 ppm and 6.27 (extra category) at 600 ppm, providing a basis for sunscreen product development.

Flavonoids and other phenolic compounds have attracted considerable attention as promising natural photoprotective agents owing to their unique chemical structures and multifunctional biological activities. Their aromatic rings with conjugated double-bond chromophore systems enable efficient absorption of ultraviolet radiation, particularly within the UV-B region, thereby reducing the amount of harmful radiation reaching the skin. In addition to direct UV absorption, flavonoids exhibit potent antioxidant activity by scavenging ultraviolet-induced reactive oxygen species (ROS), inhibiting lipid peroxidation, preserving cellular membrane integrity, and protecting DNA and structural proteins from oxidative damage. Several flavonoids have also been reported to modulate inflammatory signalling pathways and reduce the expression of pro-inflammatory mediators induced by UV exposure, providing an additional mechanism of photoprotection beyond simple ultraviolet filtering. These complementary mechanisms suggest that flavonoid-rich plant extracts may contribute to sunscreen formulations by combining ultraviolet absorption with antioxidant and anti-inflammatory properties, thereby enhancing overall photoprotective performance while potentially reducing oxidative skin damage associated with prolonged solar exposure (Korać & Khambholja, 2011; Nichols & Katiyar, 2010). Plant-derived ingredients have attracted increasing interest because they may provide photoprotective compounds with favourable biological properties; however, their efficacy and safety require scientific validation.

Spray gel dosage forms offer several practical advantages over conventional topical formulations. They enable hygienic application without direct hand contact, facilitate uniform distribution across the skin surface, improve patient convenience and compliance, and generally provide rapid drying with a pleasant cooling sensation. However, increasing the concentration of plant extracts may substantially influence critical formulation characteristics, including viscosity, sprayability, homogeneity, colour, and overall product performance. Therefore, optimisation of extract concentration is essential to achieve an appropriate balance between physicochemical properties and photoprotective efficacy.

Although previous studies have demonstrated the antioxidant activity and sunscreen potential of *Morus nigra* leaf extracts, there remains a significant research gap regarding their incorporation into spray gel formulations and the influence of different extract concentrations on both physicochemical characteristics and *in vitro* SPF values. To the best of our knowledge, comprehensive studies simultaneously evaluating formulation performance and concentration-dependent photoprotective activity of *Morus nigra* leaf extract spray gels remain scarce.

METHODS

Materials and Methods

The equipment used in this study included a 60-mesh sieve, stirring rods (Pyrex), beakers (Pyrex), a blender (Cosmos), spray bottles, porcelain dishes, a 5 mL graduated cylinder (Pyrex), filter paper (Whatman), a water bath (Faithful), universal pH indicators (Onemed), and dropping pipettes (Pyrex). Additionally, knives, mica plastic (Asahi), glass slides (Pyrex), a rotary evaporator (B-One), a UV-Vis spectrophotometer (B-One), test tubes (Pyrex), a digital balance (HWH 1002C), a Brookfield viscometer (B-One), and maceration containers were utilized.

The materials used in this research consisted of mulberry leaves (*Morus nigra* L.), 70% ethanol, 96% ethanol, and distilled water. Chemical reagents included anhydrous acetic acid, boric acid, 2N hydrochloric acid (HCl), and concentrated sulfuric acid. Carbomer 940, methylparaben, propylparaben, propylene glycol, triethanolamine (TEA), Dragendorff′s reagent and Mayer′s reagent.

Extraction

Dried mulberry (*Morus nigra* L.) leaves were pulverised and passed through a 60-mesh sieve to obtain a uniform powder. A total of 500 g of powdered simplicia was extracted with 5,000 mL of 70% ethanol (solid-to-solvent ratio of 1:10, w/v) using the maceration method. The extraction was performed at room temperature (25–28°C) for 72 h with intermittent stirring to facilitate solvent penetration and maximise the extraction of bioactive constituents. The extraction vessel was tightly covered with aluminium foil and protected from direct light throughout the maceration process to minimise photooxidative degradation of phenolic compounds and flavonoids. The residue was subsequently re-macerated using 2,500 mL of fresh 70% ethanol (1:5, w/v) for an additional 48 h. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40–45°C until a viscous extract was obtained, thereby reducing thermal degradation of heat-sensitive phytochemicals. (Anjani et al., 2025).

Charcoal Activation and Extract Color Adsorption

The charcoal activation began by crushing and grinding wood charcoal, which was then sieved through a 70-mesh screen. A 500-gram portion of the charcoal was immersed in 500 mL of a 30% KOH solution for approximately 24 hours as a chemical activator. After soaking, the charcoal was drained and dried in an oven at 150°C for one hour. For the color adsorption process, the thickened mulberry leaf extract was

weighed and heated to 100°C. Once the target temperature was reached, activated charcoal was added as an adsorbent at a concentration of 10% of the extract's weight. The mixture was stirred for 20 minutes and then filtered using filter paper and a funnel to separate the decolorized thick extract from the activated charcoal (Bakhri et al., 2021). The concentrated extract obtained after rotary evaporation was subjected to activated charcoal adsorption prior to formulation in order to reduce excessive pigmentation that could adversely affect the appearance of the spray gel. Activated charcoal, previously activated using 30% potassium hydroxide, was added at 10% (w/w) of the concentrated extract and stirred for 20 min before filtration. The decolourised extract obtained after this adsorption process was subsequently used as the active ingredient in all spray gel formulations.

Phytochemical Screening of the Extract

a. Alkaloid Test

A 0.5-gram sample of the extract was dissolved in a mixture of 1 mL 2N HCl and 9 mL distilled water, heated for 2 minutes, and then filtered. The resulting filtrate was divided into two test tubes; the first was treated with Mayer′s reagent, while the second was treated with Dragendorff′s reagent. A positive result was indicated by the formation of a yellowish-white precipitate in the first tube and a brick-red precipitate in the second tube (Abidah et al., 2025).

b. Flavonoid Test

A 0.5 grams of the extract was dissolved in 5 mL of ethanol and heated for 5 minutes. Subsequently, 0.2 grams of magnesium powder and 10 drops of concentrated HCl were added to the solution. The formation of a red color indicated a positive result for the presence of flavonoids (Abidah et al., 2025).

c. Terpenoid Test

The terpenoid content was evaluated by dissolving 0.5 grams of the extract in 1 mL of ethanol, followed by the addition of one drop of anhydrous acetic acid and two drops of concentrated H_2SO_4. A positive reaction was characterized by the appearance of a purple or orange coloration (Abidah et al., 2025).

d. Saponin Test

A 0.5 grams of the extract was dissolved in hot water and shaken vigorously for 10 seconds. The test was considered positive if a stable foam, ranging from 1 to 10 cm in height, persisted even after the addition of one drop of 2N HCl (Abidah et al., 2025).

Formulation of Mulberry Leaf Extract Sunscreen Spray Gel

The preparation of the sunscreen spray gel began by weighing all ingredients according to the designated formula. Carbopol was dispersed in distilled water in Beaker A until a transparent gel mass was formed. Simultaneously, propylene glycol, triethanolamine, methylparaben, and propylparaben were mixed in Beaker B. The mixture from Beaker B was then gradually added into Beaker A and stirred until a homogeneous base was achieved. After the base reached homogeneity, the mulberry leaf extract was incorporated according to the specific formulation. Finally, distilled water was added to the mixture to reach a total volume of 100 mL, and the preparation was homogenized once more before being transferred into spray bottles (Saputri et al., 2024). The concentrations of *Morus nigra* leaf extract (0.75%, 3.75%, and 6.75% w/v) were selected to investigate the concentration-dependent effect of the extract on the physicochemical characteristics and *in vitro* Sun Protection Factor (SPF) of the spray gel formulations. These concentrations were chosen based on preliminary formulation optimisation and previous studies demonstrating the sunscreen potential of mulberry leaf extracts. Increasing extract concentration was expected to enhance ultraviolet absorption owing to the higher abundance of flavonoids and phenolic compounds, while simultaneously allowing evaluation of possible changes in viscosity, sprayability, homogeneity, and overall formulation performance.

Table 1. Mulberry Leaf Extract Sunscreen Gel Spray Formula

No.	Ingredients	Blanko	F1	F2	F3	Uses
1.	Mulberry Leaf Extract (*Morus nigra* L.)	-	0.75 g	3.75 g	6.75 g	Active substances
2.	Carbomer 940	0.3 g	0.3 g	0.3 g	0.3 g	Gelling agent
3.	Propylene Glycol	10 mL	10 mL	10 mL	10 mL	Humectant
4.	Trietanolamine	3 drops	3 drops	3 drops	3 drops	Emulsifier
5.	Methyl paraben	0.18 g	0.18gr	0.18 g	0.18 g	Preservatives
6.	Propyl paraben	0.02 g	0.02g	0.02 g	0.02 g	Preservatives
7.	Oleum rosae	4 Drops	4 Drops	4 Drops	4 Drops	Fragrance
7.	Aquadest	100 mL	100 mL	100 mL	100 mL	Solvents

Physical Characteristic Testing of Spray Gel

a. Organoleptic Test

The color, odor, and texture of the sunscreen spray gel were visually inspected. A high-quality formulation is characterized by a clear/transparent appearance, a mild and pleasant scent free from rancidity, and a liquid texture that forms a fine mist upon application. This test was performed in triplicate (Salsabila et al., 2024).

b. Homogeneity Test

A 0.1 g sample was applied onto a glass slide and pressed with another glass slide. The preparation was observed for the presence of any coarse particles or unmixed components. A homogeneous preparation shows a uniform distribution of particles. This test was conducted in triplicate (Rizal et al., 2023).

c. pH Test

The pH value was measured by dipping a universal pH indicator into a 0.1 g sample of the spray gel. After a few moments, the color change was compared against the standard indicator. The target pH range for skin safety is 4.5–6.5. This measurement was performed in triplicate (Rizal et al., 2023). The pH measurement has been repeated using a

calibrated digital pH meter to improve measurement accuracy and precision

d. Viscosity Test

A 100 g sample was placed in a 100 mL beaker. The viscosity was measured using a Brookfield viscometer with spindle No. 3 at a speed of 30 rpm. Stable readings were recorded in triplicate. The required viscosity range for this dosage form is 500–5,000 cPs (Rizal et al., 2023).

e. Spray Pattern Test

The spray gel was discharged from a spray bottle onto a plastic film at a distance of 5 cm. The resulting spray diameter was measured using a vernier caliper. An ideal spray pattern/spreadability for a spray gel formulation ranges between 5–7 cm. This test was repeated three times (Salsabila et al., 2024).

Determination of Sun Protection Factor (SPF) Value

The SPF value was determined by weighing 1 g of each formulation, which was then mixed with 50 mL of 70% ethanol and sonicated for 15 minutes. The sonicated mixture was transferred to a 100 mL volumetric flask and diluted with 70% ethanol to the graduation mark. The solution was filtered, with the first 10 mL of the filtrate being discarded. A 100 µL aliquot of the filtered solution was then pipetted into a 25 mL volumetric flask and further diluted with 70% ethanol to the graduation mark. The absorbance of the final solution was measured using a UV-Vis spectrophotometer, which had been calibrated between 290 and 320 nm at 5 nm intervals, with 70% ethanol serving as the blank (Salsabila et al., 2024). This procedure was conducted in triplicate, and the recorded absorbance data were used to calculate the SPF values using the Mansur equation:

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

Note:
EE : Erythermal effect spectrum
I : Solar intensity spectrum
Abs : Absorbance of sunscreen product
Cf : Correction factor (=10)

Data Analysis

Data normality and homogeneity were evaluated using the Shapiro–Wilk and Levene tests, respectively. Since the assumptions of normality and homogeneity were not satisfied ($p < 0.05$), differences among the independent formulation groups were analysed using the Kruskal–Wallis test. When significant differences were observed, Dunn's multiple comparison test was performed as a post hoc analysis. Statistical significance was established at $p < 0.05$. All measurements were performed in triplicate as technical replicates using the same formulation batch.

RESULTS OF STUDY

Plant Identification

Determination is a process in research aimed at establishing the authenticity of the sample used (Sawiji & Sukmadiani, 2021). The determination was carried out at the Botany Laboratory, Faculty of Mathematics and Natural Sciences, University of Lampung. The sample used in this study was mulberry leaves (Morus nigra L.). The results of the determination showed that the plant used was correctly identified as mulberry (*Morus nigra* L.). These results officially confirm that the sample meets the taxonomic criteria as the species Morus nigra L. from the Moraceae family. This certainty of identity is essential to avoid misidentification (error in persona) of the natural material used as the research object. Therefore, the sample used in this study has been botanically standardized.

Determination Fresh-to-dry weight reduction of Mulberry Leaf Simplicia *(Morus nigra L.)*

The loss on drying test is intended to determine the maximum limit (range) of compounds lost during the drying process (Daeng et al., 2023). Sample B showed a loss on drying value of 77.3%, which is considered very high. According to the literature, this value does not meet the standard quality requirements for simplicia, which set a maximum limit of <10% (Daeng et al., 2023).

Table 2. Reduction of Fresh Leaf Weight After Dryingt of Mulberry Leaf Simplicia

Initial weight	Final weight	Shrinkage drying
7.5 kg	1.7 kg	77.3 %

The high moisture content may be attributed to several technical factors during the post-harvest process, one of which is the insufficient drying duration. Although drying was carried out using an oven at 50°C to maintain the stability of active compounds, the heating time was presumably not sufficient to remove all the strongly bound water within the thick mesophyll tissue of mulberry leaves. In addition, the reduction in weight from 7.5 kg (initial) to 1.7 kg (final) indicates a very high natural moisture content in the fresh sample, thus requiring more intensive heat energy and air circulation to achieve a constant moisture content below 10%.

Extract Preparation

The yield of mulberry leaf thick extract (*Morus nigra* L.) was calculated by comparing the amount of extract obtained with the initial simplicia used. A higher yield indicates a greater content of active compounds in the sample (Badriyah & Farihah, 2022). An extract yield is considered acceptable if it exceeds 10% (Kementerian Kesehatam Republik Indonesia, 2017).

Table 3. Extract Preparation

Simplisia	Powder Weight (gr)	Extract Weight(gr)	Yield (%)	Parameter (%)
Mulberry Leaf (*Morus nigra* L.)	500	95.843	19.**1686**	**>10**

The mulberry leaf thick extract (Morus nigra L.) was 19.1686%, thereby meeting the required standard**.** The study conducted by (Tania et al., 2025) reported an extract yield of

11.8% using 96% ethanol as the solvent. The difference in yield results may be attributed to variations in environmental growing conditions, as the samples were obtained from different locations, as well as differences in solvent concentration used during extraction. This is in line with (Rizal et al., 2023), who stated that extract yield can be influenced by several factors, including temperature, extraction time, sampling location, particle size of the simplicia, and the type of solvent used.

Phytochemical Screening

Phytochemical screening of mulberry leaves was used to qualitatively identify the presence of secondary metabolites in plants. The phytochemical screening results presented in Table 4 indicate that mulberry leaf extract contains several secondary metabolite groups, including flavonoids, saponins, and terpenoids. Flavonoids showed a positive result, as indicated by the formation of a red color, suggesting the presence of compounds that may contribute to ultraviolet absorption through chromophore structures and conjugated double bonds. Saponins and terpenoids were also detected, indicating that the extract contains additional bioactive constituents that may support the overall antioxidant properties of the formulation. However, the alkaloid result should be interpreted cautiously because the Mayer test showed a negative result, while the Dragendorff test showed a positive result. Therefore, the presence of alkaloids should be described as partially indicated and may require further confirmatory testing.

Evaluation of Mulberry Leaf Extract Spray Gel *(Morus nigra L.)*

Table 5 shows that the addition of mulberry leaf extract influenced the color and consistency of the spray gel formulations. The negative control was clear and thick, whereas F1, F2, and F3 showed progressive color changes from clear green to yellowish brown and dark brown as the extract concentration increased. This indicates that the natural pigment content of the extract contributed to the visual characteristics of the formulation. In terms of consistency, F1 and F2 were somewhat thick, while F3 was liquid, suggesting that higher extract concentration may reduce the gel consistency of the preparation. Although F3 showed the highest extract concentration, its darker color and liquid form should be considered in relation to user acceptability and the intended characteristics of a spray gel preparation.

The homogeneity test results in Table 6 show that all formulations, including the positive control, negative control, F1, F2, and F3, were homogeneous and did not show visible coarse particles. These findings indicate that the extract and excipients were uniformly dispersed within the spray gel base. Homogeneity is an important physical requirement for topical preparations because it supports uniform distribution of active compounds in each application. For spray gel formulations, homogeneous dispersion is also important to reduce the risk of nozzle blockage and ensure more consistent delivery of the active ingredient during spraying.

The pH evaluation in Table 7 shows that all formulations had a pH value of 5 across three replications. This value falls within the acceptable pH range for topical preparations and is compatible with the physiological pH of the skin. The consistency of pH values among the positive control, negative control, and extract-containing formulations suggests that the addition of mulberry leaf extract at concentrations of 0.75%, 3.75%, and 6.75% did not substantially alter the acidity of the formulation. However, because all values were identical, the authors should ensure that the pH was measured using a calibrated pH meter and report the results with appropriate precision if decimal values are available.

Table 4. Phytochemical Screening Results of Murbei Leaf

Metabolite Compounds	Reagents	Positive Results	Observation Results	Remarks
Alkaloid	Mayer	Yellowish-white precipitation	Red	-
	Dragendorff	Red sediment	Red sediment	+
Flavonoid	Magnesium powder + concentrated HCl	Red colour	Red	+
Saponin	HCl 2N	Foam formed	Foam formed	+
Terpenoid	Anhydrous acetic acid + H2SO4	Purple / Orange	Orange	+

Table. 5. Organoleptic tests

Formula	Color	Smell	Shape
Positive control	White	Typical coconut	Thick
Negative control	Clear	Rose specials	Thick
F1 (0.75%)	Clear green	Rose specials	Somewhat thick
F2 (3.75%)	Yellowish brown	Rose specials	Somewhat thick
F3 (6.75%)	Dark chocolate	Rose specials	Liquid

Table 6. Homogeneity Test

Formula	Homogenites	Results	Remarks
Positive control	Homogeneous	No rough details	Meet the requirements
Negative control	Homogeneous	No rough details	Meet the requirements
F1 (0.75%)	Homogeneous	No rough details	Meet the requirements
F2 (3.75%)	Homogeneous	No rough details	Meet the requirements
F3 (6,75%)	Homogeneous	No rough details	Meet the requirements

Table 7. pH test calibrated tool

Formula	pH			Average
	1	2	3	
Positive control	5	5	5	**5**
Negative control	5	5	5	**5**
F1 (0,75%)	5	5	5	**5**
F2 (3,75%)	5	5	5	**5**
F3 (6,75%)	5	5	5	**5**

The viscosity results in Table 8 demonstrate a decreasing trend in viscosity as the concentration of mulberry leaf extract increased. The positive control had the highest average viscosity of 4,750 cPs, followed by the negative control at 4,000 cPs. Among the extract-containing formulations, F1 showed an average viscosity of 3,083 cPs, F2 decreased to 1,750 cPs, and F3 showed the lowest viscosity at 1,000 cPs. These results indicate that increasing extract concentration may reduce the viscosity of the spray gel system. Nevertheless, all extract-containing formulations remained within the acceptable viscosity range for spray gel preparations, namely 500–5,000 cPs. The lower viscosity of F3 may improve sprayability, but it also needs to be considered carefully because an overly liquid consistency may reduce the gel-like characteristics of the formulation.

Table 9 shows that the spray dispersion diameter increased as the viscosity of the formulation decreased. The positive control and negative control produced average dispersion diameters of 2.188 cm and 4.032 cm, respectively, which were below the expected range of 5–7 cm for spray gel preparations. In contrast, F1, F2, and F3 produced average dispersion diameters of 5.592 cm, 6.607 cm, and 6.907 cm, respectively, indicating that the extract-containing formulations met the expected spray pattern requirement. The increase in dispersion diameter from F1 to F3 suggests that higher extract concentration and lower viscosity may facilitate wider spray distribution. However, the F3 formulation should still be evaluated carefully because one replication exceeded 7 cm, indicating the possibility of greater variability in spray performance.

Table 8. Viscosity Test

Formula	Speed (rpm)	Viscosity (cps)			Average	SD
		1	2	3		
Positive control	30	5,000	4,500	4,750	4,750	250
Negative control	30	4,250	4,000	3,750	4,000	250
F1 (0.75%)	30	3,000	2.750	3,500	3,083	382
F2 (3.75%)	30	1,500	2,000	1,750	1,750	250
F3 (6.75%)	30	750	1,000	1,250	1,000	250

Table 9. Spraying Pattern Test

Formula	Diameter Dispersion (cm)			Average	SD
	1	2	3		
Positive control	2.010	2.205	2.350	2.188	0.171
Negative control	4.185	3.500	4.410	4.032	0.474
F1 (0.75%)	5.940	5.405	5.430	5.592	0.302
F2 (3.75%)	6.635	6.235	6.950	6.607	0.358
F3 (6.75%)	6.980	7.165	6.575	6.907	0.302

In vitro SPF Determination

Figure 1 illustrates the in vitro SPF values of the mulberry leaf extract spray gel formulations. The SPF values increased with higher extract concentrations, indicating a concentration-dependent enhancement of photoprotective activity. F3, containing 6.75% extract, showed the highest SPF value among the tested formulations, followed by F2 and F1. This trend suggests that a greater concentration of mulberry leaf extract may increase the amount of UV-absorbing phytochemical compounds in the formulation, particularly flavonoids and phenolic compounds. However, the SPF results should be interpreted as preliminary in vitro findings. Further studies, including absorbance data presentation, photostability testing, skin irritation testing, and in vivo evaluation, are required to confirm the sunscreen efficacy and safety of the formulation.

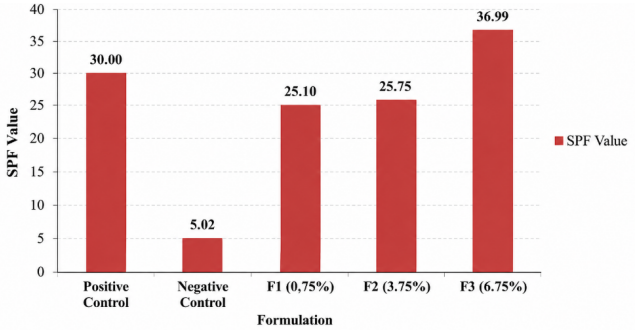

Figure 1. SPF Value of Mulberry Leaf Spray Gel

DISCUSSION

Fresh-to-Dry Weight Reduction

The loss on drying test is intended to determine the maximum limit (range) of compounds lost during the drying process (Daeng et al., 2023). Sample B showed a loss on drying value of 77.3%, which is considered very high. According to the literature, this value does not meet the standard quality requirements for simplicia, which set a maximum limit of <10% (Daeng et al., 2023).

The high moisture content may be attributed to several technical factors during the post-harvest process, one of which is the insufficient drying duration. Although drying was carried out using an oven at 50°C to maintain the stability of active compounds, the heating time was presumably not

sufficient to remove all the strongly bound water within the thick mesophyll tissue of mulberry leaves. In addition, the reduction in weight from 7.5 kg (initial) to 1.7 kg (final) indicates a very high natural moisture content in the fresh sample, thus requiring more intensive heat energy and air circulation to achieve a constant moisture content below 10%.

Phytochemical Screening

a. Alkaloid

The positive results of the alkaloids in the Dragendorff Test were characterized by the formation of brick-red deposits (Abidah et al., 2025). These deposits are potassium alkaloids. In this reaction, bismuth salts can be hydrolyzed and then create bismuth ions (BiO+), as a result bismuth nitrate is dissolved in HCl to prevent hydrolysis reactions. To preserve the ions in the solution, acid is added to this solution to shift the equilibrium to the left & Bi3+ bismuth nitrate ions react using potassium iodide to create black deposits of bismuth (III) iodide (Citra & Marpaung, 2024). The results obtained in Table 3 show brick-red deposits in mulberry leaf extract (Morus nigra L.) which indicates the presence of alkaloid compounds in the sample. In the alkaloid test with the mayer reagent, it was positive because a yellowish-white precipitate was formed. The precipitation is a potassium-alkaloid complex. In the reaction mechanism with the mayer reagent, a solution of mercury (II) chloride (HgCl2) and potassium iodide (KI) reacts giving a red precipitate. When potassium iodide is added back, potassium tetraiodomercurate (II) is formed. In the presence of nitrogen atoms that share free electron pairs, alkaloids can form covalent bonds in coordination with metal ions (Citra & Marpaung, 2024).

b. Flavonoid

The results in table.3 show positive flavonoid results. This is also in line with the research of (Tania et al., 2025) with the formation of red color in mulberry leaf samples. The purpose of adding magnesium powder and concentrated HCl is to reduce the benzopiron core in the flavonoid structure so that flavilium salts, which are colored compounds that are indicators of the presence of flavonoids in the sample (Zaini & Shofia, 2020). The reactions that occur in the flavonoid test are as follows.

c. Saponins

The results obtained in table.3 show the formation of a 1 cm high foam which indicates the presence of saponin compounds. This is reinforced by the results of research by (Tania et al., 2025) and (Saryanti et al., 2025) on saponin tests which showed the presence of foam as high as 1.5 cm in mulberry leaf samples. This compound is a type of glycoside that contains sugar molecules with 2 (two) types of agligon, namely steroids (C-27) and triterpenoids (C-30). The reagent used in the saponin test is HCI 2N. The addition of HCl 2N in the test aims to increase the polarity level, so that the hydrophilic group has a stronger bond and the foam formed is stable (Nugraha et al., 2024).

d. Terpenoid

Positive results in the terpenoid test are characterized by the formation of purple or orange (Abidah et al., 2025). The results obtained in table.3 show an orange color indicating the presence of terpenoid compounds in the sample. This is similar to (Masela, 2021) research on the terpenoid test of mulberry leaf extract which showed an orange color. The steroid test showed positive results due to the condensation or release of H_2O and the combination with carbocation. The terpenoid reaction begins with the release of the hydrogen group and its electrons, resulting in the double bond being transferred. This compound experiences resonance that acts as an electrophile and carbocation. The carbocation attack causes electrophilic addition, followed by the release of hydrogen. Then the hydrogen group and its electrons are released, as a result of which the compound experiences an extension of conjugation which shows the appearance of a red ring (Nurjannah et al., 2022). Terpenoids work as antioxidants with the primary antioxidant mechanism of action, which is able to reduce the formation of new free radicals by breaking the chain reaction and turning it into a more stable product (Ramadhan et al., 2023).

Effect of Extract Concentration on Physical Characteristics

a. Organoleptic tests

A good spray gel formulation is characterized by a clear/transparent color, a soft and pleasant aroma, free from rancid/musty odors and a liquid texture and a fine mist formed when sprayed (Salsabila et al., 2024). The results of the organoleptic test of mulberry leaf extract are shown in the table. 4 The organoleptic test in the positive control is white, has a typical coconut smell, and has a thick texture. The negative control shows a clear color and a thick texture. F1 (0.75%) has a clear green color and is slightly thick in texture. F2 (3.75%) has a yellowish-brown color and has a slightly thick texture. F3 (6.75%) has a deep brown color and has a liquid texture. All four formulations have a typical rose smell. The difference in color and texture in mulberry leaf extract spray gel preparation (*Morus nigra* L.) is caused by differences in the concentration of extracts used in the preparation.

b. Homogeneity Test

Table 7 shows the absence of particles or ingredients that have not been mixed so as to meet the requirements of a homogeneous preparation. The homogeneity of the preparation proves that the mixing and gel base selection procedures have been optimal in dispersing the extract evenly into the preparation matrix. This condition is very crucial for spray preparations to ensure a uniform distribution of active substances in each spray and prevent blockages in the bottle nozzle mechanism. When compared to the research of (Prihandini et al., 2023).

c. pH Test

A pH test is performed to determine whether the preparation's pH matches the skin's pH, which is 4.5-6.5 (Salsabila *et al.*, 2024). The pH should not be too acidic, as this can cause skin irritation, while too alkaline can cause scaly skin. The formulations of the preparations in table. 8 show positive control gel sprays, negative controls, F1 (0.75%), F2 (3.73%), F3 (6.75%) show the same pH value of 5. These values indicate that all formulations meet the requirements of a good pH range for the skin. The consistency of the pH value of 5 in all formulations shows that the addition of mulberry leaf extract with various concentrations does not significantly change the acidity degree of the preparation, which indicates good chemical stability between the active substance and the gel base. Theoretically, this pH stability is greatly influenced by the settling capacity of the base components as well as the physicochemical properties of mulberry leaf extract which tend to be stable in weak acid conditions.

d. Viscosity Test

The viscosity test measures the thickness of a liquid based on the amount of friction within the liquid. The higher the

viscosity value, the higher the viscosity (Tania et al., 2025). The ideal viscosity for a spray gel preparation is 500-5000 cPs (Rizal *et al*., 2023). Although F3 produced the highest *in vitro* SPF value, organoleptic evaluation demonstrated a darker colour and lower viscosity than the other formulations. These characteristics may reduce consumer acceptability and indicate that the formulation approaches the behaviour of a liquid spray rather than a conventional spray gel. Nevertheless, the measured viscosity (1000 cPs) remained within the recommended viscosity range for spray gel preparations (500–5000 cPs), suggesting that F3 can still be classified as a spray gel despite exhibiting a more fluid consistency. Future optimisation should focus on improving aesthetic appearance while maintaining photoprotective activity.

Based on the test results, it was found that the higher the concentration of mulberry leaf extract, the lower the viscosity value of the preparation. This phenomenon is caused by the physical characteristics of the extract, which tends to be liquid due to the use of 70% ethanol in the color adsorption process. According to (Rahmatullah et al., 2021), Carbomer 940 requires maximum water hydration to form a gel structure after neutralization by TEA. The presence of ethanol residues in the extract (especially in F3 with a concentration of 6.75%) provides a dilution effect and dehydrating properties to the polymer.

e. Spray Pattern Test

The spray pattern is one factor in determining whether the spray applicator used effectively delivers a certain amount of spray gel with each application. Factors that influence the spray pattern are viscosity and spray distance (Kresnawati et al., 2022). Spreadability testing on spray gel preparations is carried out by spraying the preparation from the bottle at a distance of 5 cm onto a sheet of plastic film. The resulting diameter is measured using a vernier caliper. The required spreadability diameter is 5-7 cm (Salsabila *et al.,* 2024).

The spraying pattern test in Table 10 was performed on positive control formulations, negative controls, F1 (0.75%), F2 (3.75%), F3 (6.75%), and resulted in an average dispersal diameter formed consecutively, which was 2.188 cm; 4.032 cm; 5.592 cm; 6.607 cm; 6.907 cm. Based on these results, the positive control resulted in the smallest average diameter of 2.188 cm, which is technically below the general requirements range of spray gel (5-7 cm). However, these results do not indicate a failure of the preparation, but rather reflect the characteristics of the preparation that more resembles a serum. This is predominantly influenced by the complexity of the active ingredients and excipients in the positive control

In vitro Photoprotective Activity

The SPF evaluation of mulberry leaf spray gel was conducted using UV-Vis spectrophotometry to determine its efficacy against ultraviolet radiation. The results showed that increasing extract concentrations led to higher SPF values, where F1 (0.75%), F2 (3.75%), and F3 (6.75%) yielded SPF values of 25.09, 25.74, and 36.98, respectively–all falling within the "ultra protection" category. This photoprotective capability is driven by secondary metabolites such as flavonoids and terpenoids, which contain chromophore groups that absorb UV energy. Statistical analysis using the Friedman and Wilcoxon tests confirmed significant differences ($p < 0.05$) among all treatment groups, proving that concentration variations directly impact radiation absorption.

A significant increase in SPF values in F1 (0.75%), F2 (3.75%), and F3 (6.75%) formulations proves that the concentration of mulberry leaf extract has a significant effect on SPF values; the higher the concentration of the extract, the greater the ability of the preparation to absorb UV radiation. This is attributed to the content of secondary metabolites in mulberry leaves, specifically flavonoid and terpenoid compounds, which have conjugated double bonds. These chromophore groups work by absorbing photon energy from UV rays and emitting it back as heat energy that does not damage the skin

A significant difference in the results of the statistical test showed that any increase in the concentration of mulberry leaf extract (from 0.75% to 6.75%) consistently significantly increased the preparation's ability to absorb UV radiation. This proves that the increase in the amount of extract is directly proportional to the increase in the number of flavonoid compounds distributed in the spray gel matrix. Thus, this statistical analysis strongly confirms that the formulation of F3 with the highest concentration (6.75%) provides the most optimal protection and is markedly different from other formulas and negative controls. Overall, this study confirms that increasing active compound levels in the formulation enhances solar radiation absorption capacity, making it a highly potential natural sunscreen agent.

Formula F3 produced the highest *in vitro* SPF value; however, organoleptic evaluation demonstrated a noticeably darker colour and a more fluid consistency than the other formulations. Although these characteristics may reduce consumer acceptability, the measured viscosity remained within the recommended viscosity range for spray gel formulations (500–5000 cPs). Therefore, Formula F3 may still be classified as a spray gel despite exhibiting properties approaching those of a liquid spray formulation. Future formulation optimisation should focus on improving aesthetic appearance while maintaining photoprotective performance.

Limitation

A limitation of the present study relates to the activated charcoal adsorption step performed prior to formulation. Although this procedure was intended to improve the aesthetic appearance of the spray gel by reducing excessive pigmentation, activated charcoal is recognised as a non-selective adsorbent capable of binding not only colouring substances but also certain phenolic compounds and flavonoids. Consequently, partial removal of these bioactive constituents may have reduced the antioxidant capacity and photoprotective activity of the extract, thereby potentially influencing the measured *in vitro* SPF values. Since the total phenolic and total flavonoid contents were not quantified before and after the adsorption process, the magnitude of this effect could not be determined. Future studies should therefore quantify these phytochemicals before and after activated charcoal treatment to better understand the relationship between adsorption, phytochemical retention, and sunscreen efficacy.

CONCLUSIONS AND RECOMMENDATION

The formulated spray gels exhibited acceptable physicochemical characteristics, including homogeneous appearance, pH within the physiological skin range, appropriate viscosity, and suitable spray patterns. Increasing extract concentration resulted in higher *in vitro* SPF values,

with F3 (6.75%) demonstrating the highest photoprotective potential. Nevertheless, these findings are limited to *in vitro* evaluation, and further stability studies, quantitative phytochemical analyses, skin irritation assessments, and in vivo or clinical investigations are required before practical application can be recommended.

DECLARATION

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The authors declare that they have no competing interests, financial or otherwise, that could have influenced the work reported in this manuscript.

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The authors declare that no artificial intelligence (AI)-assisted technology was used in the generation, analysis, interpretation of data, or preparation of the final manuscript. The authors take full responsibility for the content of this work.

Code Availability
No custom code, software, or computational algorithms were developed or used in this study. Therefore, no code is available for sharing.

Authors' Contributions
Diah Kartika Putri contributed to the study conceptualisation, methodology, supervision, and manuscript revision. Mila Naura Shalsabila, Salsabila, Riska Nur Aeni, Rena Tiyarma Sari, and Afina Rizky Ramadhani conducted the experiments, collected data, performed data analysis, and prepared the initial manuscript draft. Annajim Daskar contributed to methodology development, data interpretation, and critical revision of the manuscript. Wina Safutri contributed to study supervision, validation, and manuscript review. Vicko Suswidiantoro contributed to study design, data interpretation, supervision, critical manuscript revision, and final approval of the version to be published. All authors read and approved the final manuscript.

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