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Optimization of *Morinda citrifolia* L. Fruit Extract Gel Formulation for Antioxidant Activity and SPF Value

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Abstract

Background: *Exposure to ultraviolet (UV) radiation can cause skin damage. To mitigate this, sun protection is needed. Noni fruit (Morinda citrifolia L.) has the potential to protect the skin due to secondary metabolites, namely phenolics and flavonoids. Both possess antioxidant and UV-absorbing properties, contributing to the extract's photoprotective activity and sunscreen potential.* ***Objective:*** *This study aims to determine the optimal formula for sunscreen gel preparations using noni fruit extract, as well as the antioxidant capacity and SPF.* ***Methods:*** *The optimization used a 22-factorial design, with the tested response parameters including organoleptic properties, homogeneity, pH, spreadability, adhesiveness, and viscosity.* ***Results:*** *Based on the research conducted, the optimal formula for the gel preparation is Carbopol at 1,959% and propylene glycol at 10,515%. The noni fruit extract gel at a concentration of 10% has an antioxidant capacity with an IC50 of 941,6100 ± 27,8651 µg/mL and an SPF of 21,1702 ± 0,7166.* ***Conclusion:*** *Noni fruit extract gel at a concentration of 10% has limited antioxidant activity but has a moderate SPF value for UV protection.*

Keywords*: Morinda citrifolia L.; Factorial design; Antioxidant Activity; Sun Protection Factor.*

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INTRODUCTION

Indonesia's tropical climate results in high levels of solar radiation throughout the year, which serves as a significant environmental risk factor for skin malignancies. According to the World Health Organization (WHO) via the International Agency for Research on Cancer (IARC), Indonesia recorded 1,716 new cases of melanoma and 7,841 cases of non-melanoma skin cancer in 2022, collectively contributing to the global burden of UV-induced carcinogenesis [1]. Reactive Oxygen Species (ROS) form in the skin when chromophores absorb energy and undergo photosensitization. Uncontrolled increases in ROS can cause oxidative stress, which contributes to cell damage, inflammation, premature aging, and even carcinogenesis [2,3].

The use of sunscreen plays a crucial role in protecting the skin from UV radiation. UV radiation is grouped by wavelength: UVA (320-400 nm), UVB (280-320 nm), and UVC (100-280 nm). A sunscreen's ability to protect the skin against UV exposure is assessed by its Sun Protection Factor (SPF). The SPF indicates the skin's level of protection against sunlight, particularly UVB radiation. Sunscreen preparations must have a minimum SPF of 6 for UVB protection and a total SPF>13 for UVA protection. SPF protection levels are categorized as low, medium, high, and very high [1]. Recent research has increasingly focused on using natural ingredients as active components in cosmetics. Noni (*Morinda citrifolia* L.) is a plant commonly found in Indonesia and has been studied for various pharmacological activities. Noni fruit generally has an unpleasant taste and a pungent odor, making it less popular among the public [4]. The noni fruit contains a wide variety of compounds, including flavonoids, saponins, alkaloids, triterpenoids, and anthraquinones [4,5]. The phenolic and flavonoid compounds in noni fruit serve as antioxidants.

Recent research indicates that the antioxidant capacity of noni fruit extract has an IC50 of 22,95 µg/mL [6]. In vivo and in vitro test results indicate that a 10% noni fruit extract gel preparation has high photoprotective activity, absorbs radiation in the UVA and UVB regions, spreads optimally, reduces erythema formation, and significantly reduces Transepidermal Water Loss (TEWL) [7]. The high antioxidant capacity and potential as a photoprotective agent make noni fruit extract a potent choice for the main ingredient in sunscreen gel preparations. Based on the previous explanation, this study was conducted to optimize the sunscreen gel formula containing noni fruit extract and to determine the preparation's antioxidant capacity and SPF value. This will allow for the identification of the right gel formulation and testing its effectiveness as a potential natural active ingredient. It is hoped that the results of this study will provide a basis for developing effective sunscreen preparations from natural ingredients to protect skin from UV exposure.

MATERIALS AND METHODS

Materials

The ingredients include: noni fruit simplicia, carbopol 940, propylene glycol, triethanolamine (TEA), methyl paraben, propyl paraben, jasmine oil, ascorbic acid, titanium dioxide, distilled water, DPPH, ethanol p.a., methanol p.a., filter paper, and aluminum foil.

Tools

The tools used include: Design-Expert software, analytical digital scales, Ultrasonic extractor (Elmasonic), rotary evaporator, round-bottom flask, Buchner flask, mortar and pestle, vortex mixer, UV-Vis spectrophotometer, micropipette, NDJ-8S viscometer, pH meter, spreadability testing apparatus, and glassware.

Method

This research was a true experimental laboratory study. It was conducted from July 2025 to March 2026 in the Phytochemistry and Pharmacognosy Laboratory, Microbiology and Biotechnology Laboratory, and Pharmaceutical Laboratory at the Faculty of Pharmacy, University of Jember. The following are the research procedures.

Noni Fruit Extraction

Extraction was performed by ultrasonication with 70% ethanol (1:20 w/v) for 30 minutes. The extract was then evaporated on a rotary evaporator at 55°C until a thick extract was obtained [8].

Optimization of 10% Noni Fruit Extract Gel Formulation

Optimization of the noni fruit gel formulation was carried out to obtain an optimal gel that meets physical requirements, including organoleptic properties, homogeneity, pH, viscosity, and spreadability. Optimization was carried out using a 22 factorial design method in Design-Expert software. Optimization of the noni fruit gel formula was carried out using carbopol as a gelling agent and propylene glycol as a humectant. Carbopol was dispersed in distilled water until fully expanded, then propylene glycol and other additives were gradually added and homogenized. The noni fruit extract was added to the gel base and stirred until homogeneous, forming a stable gel [9,10].

The research began by designing a noni fruit extract gel formulation to obtain an optimal preparation. In this study, four gel formulas were prepared, with the independent variables being the concentration of carbopol and propylene glycol. The steps to obtain high and low concentrations of carbopol and propylene glycol compositions were carried out by conducting gel preparation trials. The results of the gel formula orientation are shown in Table 1.

Table 1. Results of gel formula orientation

Ingredient	F1	F (a)	F (b)	F (ab)
Morinda extract	10,0	10,0	10,0	10,0
Carbopol	0,5	2,0	0,5	2,0
Propylene glycol	5	5	15	15
TEA	1,5	1,5	1,5	1,5
Nypagin	0,2	0,2	0,2	0,2
Nypasol	0,05	0,05	0,05	0,05
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100

Physical Evaluation of Noni Fruit Gel

The physical evaluation of the noni fruit gel included organoleptic, homogeneity, pH, viscosity, and spreadability tests. The organoleptic test was performed by visually observing the gel's color, appearance, and odor to ensure that the preparation met the required characteristics [11]. Homogeneity was evaluated by placing 0.5 g of gel on a glass slide, covering it with a coverslip, and observing the presence of particles; a homogeneous gel should not contain any visible powder particles [11].

The pH of the gel was measured using a pH meter by immersing the electrode into the preparation. An acceptable topical gel should have a pH ranging from 5 to 7 to ensure skin compatibility [11,12]. Viscosity was determined using an NDJ-8S viscometer by placing 100 g of gel in a beaker and immersing the spindle completely into the sample. The viscosity value was then recorded directly from the instrument, with an acceptable range of 2,000–50,000 cP according to the United States Pharmacopeia (<912>) [13]. Finally, spreadability was assessed by placing 0.5 g of gel on a glass plate, applying a specified weight, and measuring the diameter of the spread. A spreadability value of 5–7 cm indicates good gel performance [14].

Antioxidant Capacity Test

The test was based on the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [15]. The concentration series for the test solution was 600 µg/mL, 800 µg/mL, 1000 µg/mL, 1200 µg/mL, and 1600 µg/mL. The concentration series for the positive controls was 1 µg/mL, 2 µg/mL, 4 µg/mL, 6 µg/mL, and 10 µg/mL.

Maximum Wavelength Optimization

Methanol solution (0,5 mL) was placed in a cuvette, and 2 mL of DPPH at a concentration of 40 µg/mL was added. The mixture in the cuvette was homogenized to ensure complete mixing. The absorbance of the mixture of p.a. methanol and DPPH solutions was measured over the wavelength range 400–600 nm against a blank p.a. methanol solution. The wavelength was chosen to maximize absorbance in the sample. The theoretical maximum wavelength of DPPH is 515–520 nm [15].

Optimizing Incubation Time

Test solution (0.5 mL) from one of the formulas at 1000 µg/mL was added to 2 mL of a 40 µg/mL DPPH solution. The absorbance of the solution was measured every 5 minutes for 1 hour (60 minutes) using a UV-Vis spectrophotometer at the specified maximum wavelength [19].

Determination of Percent Inhibition and IC_{50}

The test solutions (0,5 mL) and positive controls were placed in test tubes, and 2 mL of DPPH solution at a concentration of 40 µg/mL was added to each. The mixture was homogenized with a vortex mixer and incubated in the dark according to the incubation time optimization results [15,19]. The absorbance of the solution was read at the maximum wavelength determined using the wavelength optimization process. The absorbance values were entered into the following percentage-inhibition equation (1).

$$\% \text{ Inhibition} = \frac{Absorbansi\ Blanko - Absorbansi\ Sampel}{Absorbansi\ Blanko} \quad (1)$$

The measurement of antioxidant capacity in this study was based on the IC50 value, obtained from a linear regression of percentage inhibition versus extract concentration in the gel sample. The linear regression equation used was as follows (2)

$$Y = bX + a \quad (2)$$

The IC_{50} value was obtained from the X value by substituting 50 for Y, indicating 50% DPPH radical scavenging. This resulted in the following equation (3).

$$IC_{50} = \frac{50 - a}{b} \quad (3)$$

The specific categorization system classifies antioxidant potential using the following IC_{50} thresholds: very strong (<10 µg/mL), strong (10–50 µg/mL), moderate (50–100 µg/mL), medium (100–250 µg/mL), and weak (>250 µg/mL) [20].

Determination of SPF Value

A total of 0,5 g of the gel sample and the control was dispersed in p.a. ethanol and then homogenized by ultrasonication for 15 minutes. The mixture was transferred to a 10 mL volumetric flask, and p.a. ethanol was added to the mark. The resulting solution was filtered through filter paper, and the filtrate was transferred to a vial as the test solution. The absorbance of the test and control solutions was measured using a UV-Vis spectrophotometer over the wavelength range of 290–320 nm in 5 nm intervals, corresponding to the UV-B region. The absorbance data were used to calculate the Sun Protection Factor (SPF) using the Mansur method, which involves averaging the absorbance over this wavelength range [17,18]. Use Mansur's equation as follows (4).

$$SPF = CF \times \sum_{\lambda=290}^{\lambda=320} [EE(\lambda) \times I(\lambda) \times Abs(\lambda)] \quad (4)$$

Where CF = correction factor (10), EE (λ) = erythrogenic effect of radiation with wavelength λ, and Abs (λ) = spectrophotometric absorbance values at wavelength λ. The values of EE x λ are constants. The product's SPF value can determine the effectiveness of sunscreen: low (2-11), medium (12-29), and high (30-50) [21].

Data Analysis

The data analysis in this study used a factorial design to evaluate the influence of each factor and its interactions on the observed responses, using Design-Expert software. This study used a 2^2 factorial design with two factors: Carbopol concentration (X_1) and propylene glycol concentration (X_2), each at two levels (low and high). The observed responses included the gel preparation's pH, viscosity, and spreadability. The data analysis process in this study began with analyzing each factor in Design-Expert software until an optimum formula was obtained.

The antioxidant capacity IC_{50} data were compared between the gel containing 10% noni fruit extract and the positive control (ascorbic acid gel) using an independent two-sample t-test. The SPF values were compared among the gel containing 10% noni fruit extract, the positive control (titanium dioxide gel), and the negative control (gel base) using a one-way ANOVA.

RESULTS AND DISCUSSION

Gel formula optimization was conducted using a factorial design with the active ingredient, noni fruit extract, at a concentration of 10%. The results from the Design-Expert Software, using a factorial design with 22 factors, yielded four formulas for Carbopol and propylene glycol concentrations, as shown in Table 2. The sunscreen gel preparations in this study were formulated into four types: F (1), F(a), F(b), and F(ab). These four formulas were determined based on the results of an optimization using a two-factor factorial design. The factors used in this study were the gel base ingredients, namely Carbopol and propylene glycol. Each factor had two different concentration levels. Carbopol was used at low (0.5%) and high (2%) concentrations, while propylene glycol was used at low (5%) and high (15%) concentrations. Furthermore, several additional ingredients were added to the sunscreen gel preparation, namely TEA, nipagin, and nipa sol, which function as gel base components.

Four gel formulations were prepared using a 2^2 factorial design and evaluated for organoleptic properties, spreadability, and pH. The response data were analyzed in Design-Expert® to assess the effects of Carbopol 940 and propylene glycol concentrations and to identify the optimal gel formulation. The test results from Design-Expert® are presented in Table 2.

Table 2. Design-Expert software formula results with Factorial Design Method 2^2

Formula	Carbopol concentration (%)	Propylene glycol concentration (%)	pH	Viscosity (m.Pas)	Spreadability (cm)
F(1)	0,5	5	5,3±0,1865	622,3±102,1	10,5 ±1,217
F(A)	2	5	4,83±0,2728	6320±842,2	3,95±0,3047
F(B)	0,5	10	4,90±0,3154	2720±49,03	5,47±0,0865
F(AB)	2	10	4,75±0,1602	3366±386,4	4,6±0,7951

The determination of the optimum formula in this study was based on the Design-Expert software overlay plot, which shows the region of the optimum formula as predicted by the software. The graph shows two areas, including a yellow area and a grey area. The optimum formula area is shown in yellow, indicating the Carbopol and propylene glycol concentrations that meet the expected response in the test. The software selects the optimal formula by observing the desirability value, which is close to or equal to 1 and lies in the yellow area of the overlay plot, as shown in Figure 1.

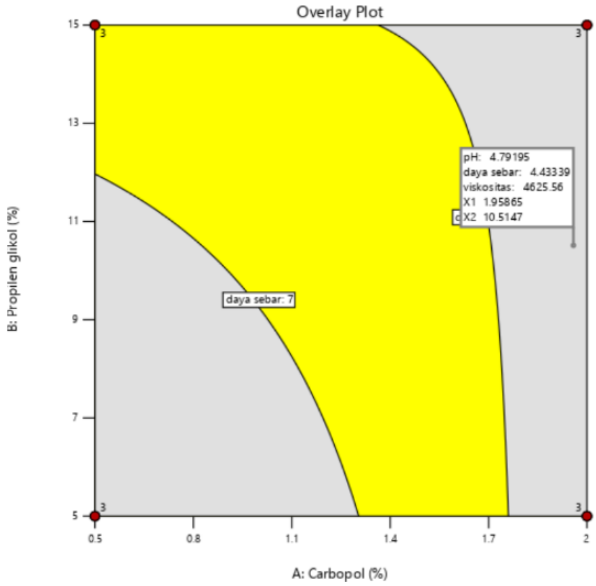

Figure 1. Overlay plot optimum formula

According to the Design-Expert software's solution, the optimal formula contained Carbopol at 1.959% and propylene glycol at 10.515%. It had a desirability value of 1 and was predicted to have a pH of 4.792, a viscosity of 4625.4 maps, and a spreadability of 4.43 cm. The physical evaluation of the preparation with the optimal formula met the criteria requirements. The physical evaluation results are shown in Table 3.

Table 3. Physical evaluation results

Parameter	Result
Form	Semi-solid gel
Color	Dark brown, typical of the extract
Odor	*Oleum jasmine* aroma
Homogeneity	Homogenous
pH	5,05
Viscosity	4575 mPas
Spreadability	5,70 cm

The optimal formula, obtained at Carbopol concentration 1,959% and propylene glycol concentration 10,515%, demonstrates that the balance between gelling agents and humectants significantly determines the physical quality of the sunscreen gel. A Carbopol concentration of approximately 2% forms a gel network strong enough to maintain the product's stability and viscosity, yet not so strong as to compromise good spreadability.

Conversely, propylene glycol at concentrations greater than 10% reduces gel stiffness by increasing the liquid phase, thereby improving spreadability and application comfort without significantly reducing viscosity. The interaction of these two components results in a product with a viscosity of 4575 mPas and a spreadability of 5,70 cm, reflecting a balance between ease of application and skin adhesion.

The resulting pH of 5,05 indicates optimal Carbopol neutralization, resulting in a stable gel structure consistent with the skin's physiological pH. Furthermore, good homogeneity indicates that the formulation system can disperse the noni fruit extract evenly throughout the gel base, which is important for ensuring consistent release of the active substance. Thus, the optimal formula not only meets the physical requirements but also indicates that the right combination of Carbopol and propylene glycol plays an important role in forming a gel system that is stable, comfortable to use, and effective at enhancing the performance of sunscreen preparations.

Gel antioxidant capacity testing was conducted using the DPPH method. The samples used in this analysis were noni fruit extract gel and ascorbic acid gel, the latter serving as a positive control. The maximum wavelength in this study was 516 nm. The optimized incubation time required to achieve stable absorbance varied by sample: 35 minutes for the sample and 2 minutes for the ascorbic acid gel positive control. Therefore, antioxidant capacity measurements were performed for each sample at the incubation time after the test solution was added to the 40 µg/mL DPPH solution.

Ascorbic acid was used as the positive control in this study because of its well-established, stable, and consistent antioxidant activity, providing a reliable reference for comparison with the antioxidant activity of gel formulations containing *Morinda citrifolia* extract [15]. In addition, the ascorbic acid gel served to verify that the antioxidant assay was functioning properly and capable of generating valid and reliable data [16]. The antioxidant activity of each formulation and the positive control was expressed as the half-maximal inhibitory concentration (IC_{50}). The IC_{50} values are presented in Figure 2, which illustrates the comparative antioxidant activity of the tested formulations. Data are presented as mean ± SD. Mean values followed by different superscript letters indicate statistically significant differences among the study groups ($p < 0.05$).

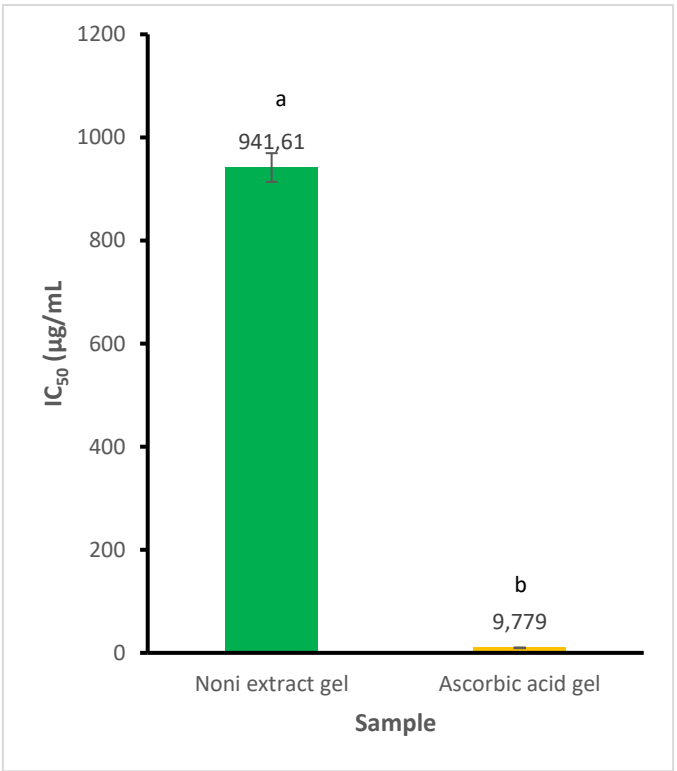

Figure 2. Antioxidant activity of noni extract gel and ascorbic acid gel expressed as IC_{50} values. Data are presented as mean ± SD. Bars with different lowercase letters indicate statistically significant differences between groups ($p < 0.05$).

IC_{50} values were analyzed using an independent-samples t-test (Figure 2). The results indicated that the antioxidant capacity, as measured by the sample's IC_{50}, differed significantly from that of the positive control. Noni fruit extract was reported to have an IC_{50} of 2,784 µg/mL [8]. However, in this study, an IC_{50} of

941,6100 µg/mL was obtained, which is much higher than that reported in the previous study. This difference is thought to be caused by differences in antioxidant capacity testing methods, especially the ratio of DPPH to sample volumes. This study used a DPPH sample ratio of 4:1 (2 mL DPPH and 0,5 mL sample). Conversely, another investigation used a 5:4 ratio (100 µL DPPH and 80 µL sample), resulting in a higher antioxidant capacity attributed to the greater proportion of DPPH relative to the sample volume [8].

The limited antioxidant capacity of noni fruit extract gel may be due to instability in the gel formulation. This study focused on evaluating antioxidant capacity in topical dosage forms, base characteristics, formula stability, and the extract's release capacity from the base [23]. These conditions can degrade bioactive compounds and reduce the release of active substances, thereby contributing to the gel preparation's low antioxidant capacity.

The observed reduction in antioxidant capacity following formulation is corroborated by previous studies, which reported that a fruit extract with an IC_{50} of 146.73 µg/mL exhibited a lower capacity of 104.659 µg/mL when incorporated into a 0.61% gel preparation [24]. Similarly, weak antioxidant performance has been noted in other gel-based delivery systems; for instance, formulations tested at concentrations of 1%, 2%, and 3% yielded IC_{50} values of 455.21, 374.08, and 330.30 µg/mL, respectively. These relatively high IC_{50} values are typically attributed to the sub-optimal release of bioactive substances from the gel matrix during the DPPH-based antioxidant assay [25].

The antioxidant capacity of natural products is closely linked to their total phenolic and flavonoid content. Phenolic compounds have high antioxidant capacity due to their redox properties, which facilitate the donation of electrons or hydrogen atoms [26]. Furthermore, optimizing extraction kinetics for noni fruit has shown that these bioactive constituents play a pivotal role in enhancing overall antioxidant performance [27]. Recent high-precision phytochemical analyses using LC-MS Q-ToF have identified a broad spectrum of secondary metabolites, including alkaloids and glycosides, which underpin the plant's diverse pharmacological and antioxidant activities. The antioxidant capacity of noni fruit is specifically linked to prominent compounds such as rutin, caffeic acid, quercetin-3-glucoside, and kaempferol [28].

SPF values were measured using the Mansur method with a UV-Vis spectrophotometer. The samples tested were noni fruit extract gel, titanium dioxide gel (positive control), and gel base (negative control). Titanium dioxide was used as the positive control because it effectively absorbs and protects the skin from UVB and UVA radiation and is often used as a reference for comparing SPF values in sunscreen formulations [17]. The SPF values for each sample are shown in Figure 3.

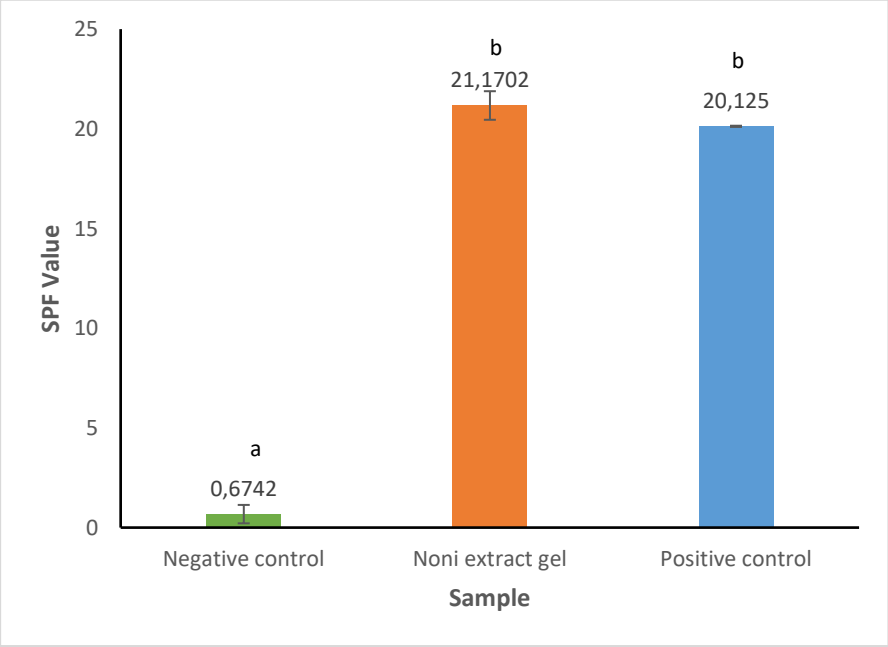

Figure 3. Sun protection factor (SPF) values of the negative control, noni extract gel, and positive control. Data are presented as mean ± SD. Different lowercase letters above the bars indicate statistically significant differences between groups ($p < 0.05$).

The Sun Protection Factor (SPF) was determined through in vitro testing, grounded in the established concept of the Minimal Erythema Dose (MED), which is the ratio of the UV radiation energy required to produce erythema on protected skin to that required on unprotected skin [29]. An SPF of approximately 21 indicated

that protected skin required twenty-one times the UV energy to achieve the same level of erythema as unprotected skin. This photoprotective capacity is fundamentally linked to the specific profile of phenolic compounds and flavonoids identified in noni fruit, which serve as intrinsic natural UV filters [30]. Furthermore, these findings are corroborated by recent meta-analytical evidence demonstrating that consistent sunscreen application significantly reduces the incidence of sunburn, confirming that higher SPF values are directly proportional to enhanced UVB protection and the duration of tolerated sun exposure [31].

The one-way ANOVA showed a significance value of $p < 0,001$ ($p < 0,05$), indicating a statistically significant difference in SPF values among the noni fruit extract gel, positive control, and negative control groups. The Tukey HSD post hoc test showed that the SPF of the extract gel was significantly different from that of the negative control ($p < 0,001$), indicating that adding noni fruit extract to the gel preparation increased its protective activity against UV radiation. There was no significant difference between the extract gel and the positive control ($p = 0,090$; $p > 0,05$).

The substantial SPF observed in the noni fruit extract gel suggests that its primary bioactive constituents, namely flavonoids and phenolic compounds, provide effective protection against ultraviolet radiation, specifically UVB. These polyphenolic compounds absorb UVA and UVB radiation and exhibit significant antioxidant activity, thereby enhancing the photoprotective effect of natural sunscreen formulations [32]. Computational and molecular analyses further suggest that bioactive compounds, including kaempferol and various phenolic derivatives in the extract, have considerable potential as radiation protection agents owing to their inherent antioxidant and chemopreventive properties [33]. Moreover, specific iridoids identified in noni fruit, such as deacetylasperulosidic acid, have been shown to inhibit UVB-induced activation of the transcription factor c-Jun N-terminal kinase (AP-1), thereby providing essential cellular protection against oxidative damage and mitigating the inflammatory cascade [34].

Despite identifying an optimal formula, this study acknowledges several limitations that warrant further investigation. First, photoprotective and antioxidant activities were assessed solely in vitro, which may not fully reflect the preparation's biological efficacy and safety when applied to human skin under real-world conditions. Second, the limited antioxidant capacity observed (IC_{50} 941.61 µg/mL) suggests that the current Carbopol-based matrix may not have enabled optimal release of the extract's phenolic and flavonoid constituents, or it may have failed to protect these unstable secondary metabolites from degradation during formulation. Additionally, this research focused on immediate physical characteristics and did not include long-term stability trials or in vivo Minimal Erythema Dose (MED) evaluations to validate its clinical potential. Addressing these constraints through advanced delivery systems and human trials will be essential to bridge the gap between laboratory optimization and the development of a commercially viable natural sunscreen product.

CONCLUSION AND SUGGESTION

This study optimized a sunscreen gel containing 10% Morinda citrifolia L. fruit extract using a 2^2 factorial design, identifying 1.959% Carbopol 940 and 10.515% propylene glycol as the optimal combination. The resulting preparation exhibited excellent physical properties, including a pH of 5.05, a viscosity of 4575 mPas, and a spreadability of 5.70 cm, all of which met established quality standards for topical applications. Furthermore, the optimized gel provided moderate photoprotection with an SPF of 21.17 ± 0.71, primarily attributable to UV-absorbing flavonoids and phenolic compounds in the extract. However, antioxidant capacity was limited, with an IC_{50} of 941.61 ± 27.87 µg/mL, likely due to suboptimal release of bioactive constituents from the gel matrix or instability of secondary metabolites during formulation.

Building on these findings, future research should focus on improving the delivery and stability of bioactive compounds in the gel system to overcome the observed limitations in antioxidant performance. Specifically, investigating alternative gelling agents or incorporating penetration enhancers could increase the release rate of antioxidant constituents from the matrix. Furthermore, exploring advanced delivery systems, such as nano-emulsions or lipid vesicles, is recommended to protect the extract’s secondary metabolites from degradation. To validate the practical application of this natural sunscreen, subsequent studies should also include in vivo efficacy trials to determine the Minimal Erythema Dose (MED) and long-term stability testing to ensure the formulation’s integrity over an extended shelf life.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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