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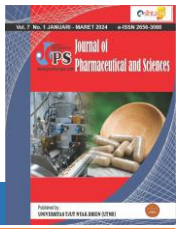

Evaluation of Skin Irritation Potential of a Butterfly Pea (*Clitoria ternatea*) Extract Sunscreen Spray Using the Patch Test Method

Uji Iritasi Kulit Sediaan Sunscreen Spray yang Mengandung Ekstrak Bunga Telang (*Clitoria ternatea*) dengan Metode Patch Test

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Abstract

Background: Butterfly pea flower (*Clitoria ternatea*) contains high levels of anthocyanins with potent antioxidant activity, making it a promising natural ingredient for sunscreen formulations. However, the skin safety of sunscreen spray containing this extract must be evaluated before cosmetic application. **Objective:** This study aimed to evaluate the skin irritation potential of sunscreen spray formulations containing butterfly pea flower extract using the patch test method. **Methods:** A patch test was conducted on 15 male Wistar rats. The test materials included sunscreen spray formulations with butterfly pea flower extract at concentrations of 1% (F1), 2% (F2), and 3% (F3). A base formulation without extract (F0) served as a negative control, and sodium lauryl sulfate served as a positive control. Erythema and edema scores were observed at 24, 48, and 72 hours after application. **Results:** The mean erythema and edema scores for formulations F1, F2, and F3 were 0.0 ± 0, indicating no signs of skin irritation in all tested groups. The positive control group showed slight erythema (score 2.33 ± 0.5), confirming the validity of the test method. **Conclusion:** Sunscreen spray formulations containing butterfly pea flower extract at concentrations of 1%, 2%, and 3% did not induce skin irritation in male Wistar rats under the patch test conditions. These findings support the potential of butterfly pea flower extract as a safe natural ingredient for sunscreen products.

Keywords*: *Butterfly Pea Flower; Sunscreen Spray; Irritation Test; Patch Test Method.

Abstrak

Latar Belakang: Bunga telang (*Clitoria ternatea*) mengandung antosianin tinggi dengan aktivitas antioksidan yang kuat, sehingga berpotensi sebagai bahan alami dalam sediaan tabir surya. Namun, keamanan terhadap kulit dari sediaan *sunscreen spray* yang mengandung ekstrak ini perlu dievaluasi sebelum aplikasi kosmetik. **Tujuan:** Penelitian ini bertujuan untuk mengevaluasi potensi iritasi kulit dari sediaan *sunscreen spray* yang mengandung ekstrak bunga telang menggunakan metode *patch test*. **Metode:** Uji *patch test* dilakukan pada 15 ekor tikus Wistar jantan. Bahan uji berupa sediaan *sunscreen spray* dengan konsentrasi ekstrak bunga telang 1% (F1), 2% (F2), dan 3% (F3). Formulasi dasar tanpa ekstrak (F0) digunakan sebagai kontrol negatif dan natrium lauril sulfat sebagai kontrol positif. Skor eritema dan edema diamati pada 24, 48, dan 72 jam setelah aplikasi. **Hasil:** Skor rata-rata eritema dan edema pada formulasi F1, F2, dan F3 adalah 0,0 ± 0, yang menunjukkan tidak terjadi tanda-tanda iritasi kulit pada semua kelompok uji. Kelompok kontrol positif menunjukkan eritema ringan (skor 2,33 ± 0,5), yang mengonfirmasi validitas metode uji. **Kesimpulan:** Sediaan *sunscreen spray* yang mengandung ekstrak bunga telang pada konsentrasi 1%, 2%, dan 3% tidak menyebabkan iritasi kulit pada tikus Wistar jantan berdasarkan metode *patch test*. Hasil ini mendukung potensi ekstrak bunga telang sebagai bahan alami yang aman untuk produk tabir surya.

Kata Kunci: Bunga Telang; Sunscreen Spray; Uji Iritasi; Patch Test Method.

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Introduction

Excessive exposure to ultraviolet (UV) radiation can cause various skin problems, including premature aging, DNA damage, and an increased risk of skin cancer [1]. Therefore, the use of sunscreen is strongly recommended as a preventive measure to protect the skin from the harmful effects of UV radiation. Currently, numerous studies have focused on developing sunscreen formulations based on natural ingredients to minimize the adverse effects associated with synthetic chemical compounds commonly used in conventional sunscreens [2]. Butterfly pea flower *(C. ternatea)* is a plant with considerable potential in cosmetic and health applications. The extract of butterfly pea flower is known to be rich in anthocyanins, which function as potent antioxidants [3]. In addition to their antioxidant properties, anthocyanins have been reported to possess the ability to absorb ultraviolet (UV) radiation, which may contribute to photoprotective effects on the skin. Through their antioxidant activity, anthocyanins can also neutralize reactive oxygen species (ROS) generated by UV exposure, thereby reducing oxidative stress and preventing cellular damage. These properties suggest that *C. ternatea* extract may serve as a promising natural ingredient in sunscreen formulations [4].

Skin irritation assessment is based on the principle that substances inducing inflammatory reactions in the skin may indicate potential irritation in humans [5], [6]. Therefore, experimental animals such as rats or rabbits are frequently used as models to predict skin irritation responses in humans [6]. To ensure the safety of sunscreen formulations containing butterfly pea flower extract, skin irritation testing is a crucial step. Skin irritation studies are commonly conducted using male Wistar rats (*Rattus norvegicus*) [7]. Male Wistar rats are selected due to their sensitive skin, hormonal stability, and skin responses to chemical irritants that closely resemble those of humans [8].

This study aims to evaluate the skin irritation potential of a sunscreen spray product containing butterfly pea flower extract. The methodology involves topical application of the sunscreen formulation onto the dorsal skin of rats over a specified period, followed by observation and measurement of irritation indicators such as erythema and edema [9]. The findings of this study are expected to provide valuable information regarding the safety of sunscreen spray formulations containing butterfly pea flower extract prior to further development and potential application in humans.

Experimental Section

This study was conducted using the patch test method through animal testing to evaluate the skin irritation potential of sunscreen spray formulations containing butterfly pea flower extract. Male Wistar rats (*Rattus norvegicus*) were used as experimental animals. The experiment was carried out in 2024 at the Pharmacology Laboratory, Universitas Ahmad Dahlan, Yogyakarta, Indonesia. All experimental procedures were approved by the Ethics Committee of Universitas Ahmad Dahlan, with ethical clearance number 022404074. Prior to the experiment, the dorsal skin of the rats was shaved carefully to provide a clean application area. The test materials consisted of 0.5 mL of sunscreen spray formulations containing butterfly pea flower extract at concentrations of 1% (F1), 2% (F2), and 3% (F3). A base formulation without extract (F0) was used as the negative control. As the positive control, 0.5 mL of a 10% sodium lauryl sulfate (SLS) solution prepared in distilled water was applied. Each test material was applied topically to the shaved dorsal skin of the rats using the patch test method and covered with sterile gauze and adhesive tape to maintain contact with the skin for 24 hours. After the exposure period, the patches were carefully removed and any remaining sample residue on the skin surface was gently cleaned using moist cotton soaked in warm distilled water to

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avoid additional mechanical irritation [10]–[13]. Skin irritation responses were evaluated by observing the appearance of erythema and edema at 24, 48, and 72 hours after patch removal. The severity of skin reactions was recorded according to the standard scoring system for erythema and edema. Additional observations were conducted up to 14 days to monitor any delayed irritation responses. No signs of erythema or edema were observed during the extended observation period in all treatment groups except the positive control [14]–[16].

Materials and Apparatus

Butterfly pea flower extract *(C. ternatea)*, ethylhexyl methoxycinnamate, propylene glycol, cetyl alcohol, citric acid, glycerin, triethanolamine (TEA), Span 80, Tween 80, phenoxyethanol, fragrance, and distilled water were used in this study [17].

Formulation of Sunscreen Spray

The sunscreen spray formulations presented in Table 1 were modified from the study by Ariyanti et al. (2022) and prepared in several concentrations [18]. The sunscreen spray was formulated by preparing the oil phase (cetyl alcohol, phenoxyethanol, and Span 80) and the aqueous phase (one-third of the distilled water, glycerin, Tween 80, and TEA) separately. Both phases were heated in a water bath to 70 °C and then gradually added to the oil phase with continuous stirring [19].

Ethylhexyl methoxycinnamate was added to the mixer, followed by the addition of Butterfly Pea Flower Extract (BPE), and the mixture was stirred for 1 minute. Four formulations were prepared using the same procedure with extract concentrations of 0%, 1%, 2%, and 3%. The remaining distilled water was added to adjust the final volume, and the sunscreen spray was allowed to cool to room temperature before being transferred into spray bottles.

Table 1. Formula for sunscreen spray with butterfly pea flower extract.

Material	Concentration (%)			
	F0	F1	F2	F3
BPE	0	1	2	3
Ethylexy methoxycinimate	10	10	10	10
Propeline glycol	10	10	10	10
Cetyl alcohol	5	5	5	5
Span 80	2	2	2	2
Tween 80	5	5	5	5
Glycerin	10	10	10	10
TEA	1	1	1	1
Stearic acid	2,5	2,5	2,5	2,5
Phenoxyethanol	1	1	1	1
Fragrance	1	1	1	1
Aquadest (ad)	100	100	100	100

Skin Irritation Test

This study was conducted to evaluate the potential skin irritation effects of sunscreen spray formulations containing butterfly pea flower extract. A total of 15 male Wistar rats (*Rattus norvegicus*) were randomly assigned into five experimental groups, with each group consisting of three rats [20]. Group 1 received a sunscreen spray formulation containing 1% butterfly pea flower extract, Group 2 received a formulation containing 2% extract, and Group 3 received a formulation containing 3% extract. Meanwhile, Group 4 was treated with the base formulation without the addition of butterfly pea flower extract (0%) and served as the negative control. Group 5 was treated with sodium lauryl sulfate (SLS) as the positive control to induce skin irritation responses.

This study received ethical approval from the Research Ethics Committee of Universitas Ahmad Dahlan for animal experimentation (Approval No. 022404074). Male Wistar rats aged 2–3 months and weighing 150–250 g were used in accordance with the patch test method. Prior to the experiment, the animals were acclimatized for 7 days under laboratory conditions [21].

The irritation test was initiated by shaving the dorsal area of the rats (2 × 3 cm), followed by a 24-hour resting period. Subsequently, 0.5 mL of each test formulation (F1, F2, and F3), the base formulation (F0) as a negative control, and sodium lauryl sulfate as a positive control were applied topically to the designated skin areas [22]. The application sites were covered with a non-irritating occlusive dressing and left for 4 hours.

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After removal of the dressing, residual samples were gently cleaned from the skin. Skin reactions were observed and scored at 24, 48, and 72 hours after exposure [20], [23]. The animals were further monitored for up to 14 days to assess any delayed irritation effects.

Data Analysis

Skin irritation test results were expressed as mean ± standard deviation (SD). Skin irritation assessment was performed using the Draize scoring method based on erythema and edema scores. The data were presented descriptively to evaluate the skin irritation potential of each formulation.

Results and Discussion

Observations were conducted to evaluate signs of skin irritation, including erythema (redness) and edema (swelling), based on the Draize scoring scale. According to the Draize scoring system, irritation responses are classified as follows: 0 = no erythema or edema, 1 = very slight, 2 = slight, 3 = moderate, and 4 = severe irritation. The clinical observation results indicated that no significant signs of irritation were observed in the groups of rats treated with formulations F0, F1, F2, and F3. In contrast, the positive control group treated with sodium lauryl sulfate (SLS) exhibited signs of irritation characterized by erythema, although no edema was observed. The interpretation of skin irritation indicators is presented in Table 2.

Observations were performed at 24, 48, and 72 hours after application. Figure 1 illustrates the condition of the experimental animals at 72 hours. Figure 1(a) shows Group 1 treated with sunscreen spray containing 1% butterfly pea flower extract (BPE), in which no erythema or edema was observed after 72 hours. Figure 1(b) presents Group 2 treated with sunscreen spray containing 2% BPE, which also showed no signs of erythema or edema. Similarly, Figure 1(c) depicts Group 3 treated with sunscreen spray containing 3% BPE, with no observable erythema or edema after 72 hours. Figure 1(d) represents Group 4 treated with the base formulation (0% BPE), which did not show any signs of skin irritation. In contrast, Figure 1(e) shows Group 5 treated with 10% SLS, which exhibited visible erythema without edema.

The positive control group treated with SLS showed an erythema score of 2.33 ± 0.5, which according to the Draize classification corresponds to slight irritation, even though edema was not observed. This finding confirms that the experimental model was able to detect irritation responses, thereby validating the reliability of the skin irritation test method used in this study.

The results of this study demonstrated that no significant skin irritation occurred following topical application of the test formulations for up to 72 hours, including during the 14-day observation period. These findings are consistent with previous studies that employed the patch test method using male Wistar rats. A study conducted by Kong et al. (2016) reported no significant skin irritation following 48 hours of application of herbal-based cosmetic materials using a similar experimental design. These results support the findings of the present study, indicating that the tested formulations did not induce skin irritation in Wistar rats [24]. A comparable study by Saputri et al. (2024), which evaluated the skin irritation of natural-based sunscreen formulations using rabbits as experimental animals, also reported no signs of irritation after 72 hours of observation [20].

The absence of irritation observed in this study may be related to the bioactive compounds present in butterfly pea flower extract, particularly anthocyanins. Anthocyanins are widely reported to exhibit antioxidant and anti-inflammatory activities [3]. The anti-inflammatory properties of these compounds may help suppress inflammatory responses in the skin, thereby reducing the potential for irritation reactions such as erythema or edema. In addition, several excipients used in the formulation, such as glycerin and propylene glycol, are commonly used humectants in cosmetic products and are generally considered safe and non-irritating to the skin when used within appropriate concentrations. These factors may contribute to the absence of irritation observed in the tested sunscreen spray formulations.

Based on the series of skin irritation tests conducted on sunscreen spray formulations containing butterfly pea flower extract (Figure 1), it can be concluded that the formulations were safe based on skin irritation evaluation in experimental animals. This study has several limitations that should be considered. First, the limited sample size may affect the generalizability of the results. In addition, individual variability among the animals and environmental conditions may have influenced the outcomes. Furthermore, this study was limited to skin irritation testing in experimental animals; therefore, the safety of the formulation in human skin, particularly in individuals with sensitive skin, cannot yet be fully confirmed. Further studies involving

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clinical trials in human subjects are necessary to validate the safety and applicability of this formulation for cosmetic use [7].

Table 2. Assessment of Skin Irritation Signs Using the Draize Scoring Scale

Group test	Erythema score	Edema score	Description
Group 1	0,0 ± 0	0,0 ± 0	Not irritating
Group 2	0,0 ± 0	0,0 ± 0	Not irritating
Group 3	0,0 ± 0	0,0 ± 0	Not irritating
Group 4	0,0 ± 0	0,0 ± 0	Not irritating
Group 5	2,33±0,5	0,0±0	Slightly irritating

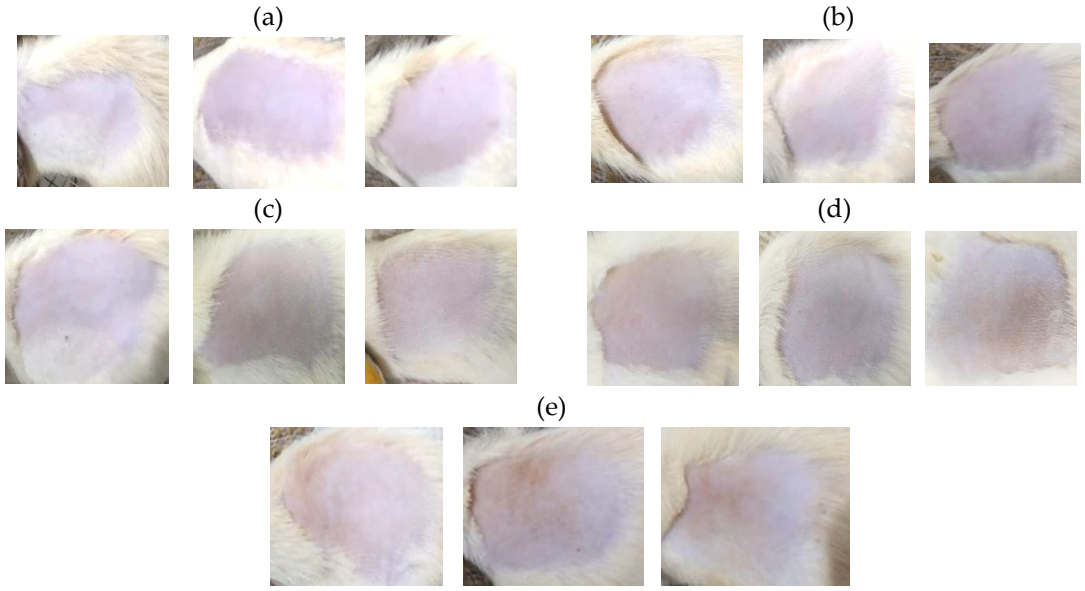

Figure 1. Results of the skin irritation test in male Wistar rats. (a) Group 1 treated with sunscreen spray containing 1% butterfly pea flower extract (BPE); (b) Group 2 treated with sunscreen spray containing 2% BPE; (c) Group 3 treated with sunscreen spray containing 3% BPE; (d) Group 4 treated with the base sunscreen spray formulation (0% BPE); and (e) positive control treated with 10% sodium lauryl sulfate (SLS).

Conclusions

Butterfly pea flower *(C. ternatea)* extract was successfully formulated into sunscreen spray preparations at concentrations of 1%, 2%, and 3%. Based on the skin irritation test conducted on male Wistar rats using the patch test method, the sunscreen spray formulations containing butterfly pea flower extract did not induce skin irritation, as indicated by the absence of erythema and edema in the tested groups. These findings suggest that the developed formulations are safe for topical application under the tested experimental conditions. The results of this study indicate that butterfly pea flower extract has potential to be further developed as a natural ingredient in sunscreen formulations. However, this study was conducted with a limited sample size and only involved experimental animals. Therefore, further studies with larger sample sizes, as well as additional evaluations such as photoprotective efficacy testing against ultraviolet radiation and clinical trials in human subjects, are necessary to comprehensively assess the safety and effectiveness of this formulation for potential cosmetic applications.

Conflict of Interest

The authors affirm that they have no competing interests or conflicts of interest related to the publication of this manuscript.

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