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Effect of Topical Dayak Onion (*Eleutherine palmifolia*) Cream on Epidermal Thickness in UVB-Exposed Male Wistar Rats: An Experimental Study

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ABSTRACT

Background: Exposure to ultraviolet B (UVB) radiation, particularly in tropical regions like Indonesia, can cause skin damage characterized by increased epidermal thickness due to keratinocyte hyperproliferation, contributing to photoaging. Natural photoprotective agents rich in antioxidants such as Dayak onion (*Eleutherine palmifolia*) extract are expected to mitigate these effects.
Objective: This study aims to evaluate the effect of applying Dayak onion extract cream at varying concentrations on epidermal thickness of UVB-induced male Wistar rat skin.
Methods: : This study was a true experimental study with a post-test only control group design. A total of 36 male Wistar rats were randomly divided into four groups: a control group (given placebo cream) and three treatment groups given Dayak onion extract cream at concentrations of 10%, 15%, and 20%. UVB exposure was carried out every two days for 30 days, along with application of the cream before and after UVB radiation. Epidermal thickness was analyzed through histopathological examination using Hematoxylin-Eosin staining and observed under 400x magnification.
ResultsStatistical analysis showed significant differences ($p < 0,05$) in epidermal thickness between the control group and the treatment groups. The mean epidermal thickness in the treatment groups was lower than that of the control group, with the greatest reduction observed in the 20% concentration group, followed by the 15% and 10% groups. There was no significant difference between the 15% and 20% concentrations.
Conclusion: Application of Dayak onion extract cream is effective in reducing epidermal thickness, with a concentration of 20% providing optimal protective effects. These findings support the potential of Dayak onion as a natural photoprotective agent.

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1. Introduction

An adequate amount of ultraviolet (UV) radiation is needed by the human body, particularly for the production of vitamin D, which plays a vital role in immune regulation and disease prevention.[1] However, excessive sun exposure has been shown to negatively affect human skin.[2] Prolonged UV exposure can lead to various skin problems, such as premature aging, sunburn, erythema, hyperpigmentation, and even skin cancer, particularly when the skin lacks protection.[3,4]

Among the different types of UV radiation, ultraviolet B (UVB) is particularly harmful due to its ability to penetrate the epidermis and induce direct DNA damage.[5] The epidermis serves as the outermost barrier of the body and is directly affected by environmental stimuli, including UVB radiation. UVB exposure stimulates epidermal keratinocyte proliferation through activation of the epidermal growth factor receptor (EGFR), leading to an increase in epidermal thickness.[6,7] This condition, known as epidermal hyperplasia or hyperkeratosis, is one of the hallmarks of skin photoaging.[8] Over time, thickening of the epidermis may result in wrinkling, loss of elasticity, rough skin texture, and a heightened risk of developing skin cancer.[9]

One method of photoprotection to shield the skin from harmful UVB rays is the use of sunscreen, which function by either absorbing or reflecting ultraviolet radiation.[10,11] However, many synthetic chemical sunscreens may cause adverse side effects such as allergic reactions, mild toxicity, and possibly an increased risk of skin cancer.[12] Therefore, there is growing interest in using natural and plant-based materials rich in bioactive compounds as safer alternatives with fewer side effects.[13,14]

One such group of bioactive compounds is flavonoids, known for their strong antioxidant properties and ability to scavenge free radicals[15]. Phenolic compounds, particularly flavonoids, are promising candidates for natural sunscreens

due to their chromophore groups, which can absorb UVA and UVB radiation and reduce its intensity on the skin.[16]

Eleutherine palmifolia (commonly known as Dayak onion) is a plant that naturally thrives under sunlight and has good adaptability to local climates and soil types. It possesses various therapeutic properties including antimicrobial, antiviral, anti-inflammatory, antihypertensive, antidiabetic, anti-dermatophytic, anti-melanogenesis, and anticancer activities. In Indonesia, it has been used by locals as a traditional herbal remedy.[17–19] The plant exhibits strong antioxidant activity due to the abundance of secondary metabolites such as flavonoids, aldehydes-ketones, carboxylic acids, glycosides, tannins, phenols, carbohydrates, and proteins. It also contains naphthoquinone derivatives like elecenacin, eleutherine, eleutherol, and eleutherinon.[19] These bioactive compounds can be isolated through extraction procedures.[20]

Previous studies have demonstrated that the ethanolic extract of *Eleutherine palmifolia* exhibits strong antioxidant activity, with an IC50 value of 41.46 mg/L, indicative of high antioxidant potency. This strong antioxidant activity suggests that *Eleutherine palmifolia* extract cream may be a promising solution for mitigating the adverse effects of UV radiation on the skin.

This background underlies the current study, which aims to investigate the effect of topical application of *Eleutherine palmifolia* (Dayak onion) cream on the histopathological features of epidermal thickness in UVB-irradiated male Wistar rats. Wistar rats were chosen due to their genetic and physiological similarities to humans, enabling relevant results without endangering human health..

2. Methods

This study employed a true experimental design using a post-test only control group design involving animal models. The overall sample design used in this study is illustrated in Figure 1.

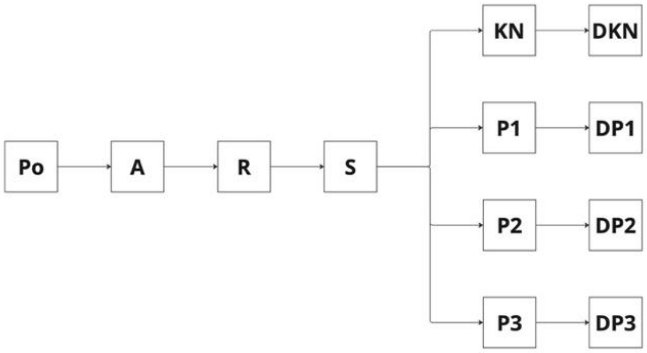

Figure 1. Research Sample Design

Description:
Po : Population of male white Wistar rats
A : Acclimatization for 7 days
R : Randomization. A total of 36 male white Wistar rats were selected from the population and randomly divided into four groups.
S : Sample. The sample consisted of male white Wistar rats that met the inclusion criteria.
KN : Control group, received placebo cream 20 minutes before and 4 hours after UVB irradiation (60 minutes every two days).
P1 : Treatment group 1, received 10% *Eleutherine palmifolia* extract cream 20 minutes before and 4 hours after UVB irradiation (60 minutes every two days).
P2 : Treatment group 2, received 15% *Eleutherine palmifolia* extract cream 20 minutes before and 4 hours after UVB irradiation (60 minutes every two days).
P3 : Treatment group 3, received 20% *Eleutherine palmifolia* extract cream 20 minutes before and 4 hours after UVB irradiation (60 minutes every two days).
DKN : Post-test data on the epidermal thickness of male white Wistar rats in the KN group
DP1 : Post-test data on the epidermal thickness of male white Wistar rats in the P1 group
DP2 : Post-test data on the epidermal thickness of male white Wistar rats in the P2 group
DP3 : Post-test data on the epidermal thickness of male white Wistar rats in the P3 group

The population of this study is Male Wistar rats housed at the Biology Laboratory of Universitas Negeri Semarang that met inclusion criteria and none of the exclusion criteria. The inclusion criteria were healthy, male Wistar rats aged 8–12 weeks with weight that ranged from 200–250 grams. The exclusion criteria were sick rats or rats with skin or anatomical abnormalities. Criteria for dropout included rats escaping from their cages or rats showing altered behavior (e.g., loss of appetite, lethargy). The sampling technique used was probability sampling using simple random sampling by numbering the rats and randomizing their group allocation. Sample Size was calculated using Federer's formula (1991) for t = 4 groups:

$$(n-1)(t-1) \geq 15$$
$$(n-1)(4-1) \geq 15$$
$$(n-1).\,3 \geq 15$$
$$(n-1) \geq 5$$
$$n \geq 6$$

n: required sample size
t: number of groups

Based on this formula, the calculated number of samples per group (n) is six (6) rats, resulting in a total of twenty-four (24) rats overall.
Including a 30% dropout rate:

$$n' = \frac{n}{1-f}$$
$$n' = \frac{6}{1-0.3}$$
$$n' = \frac{6}{0.7}$$
$$n' = 8{,}6 \sim 9$$

n' : number of samples per group after adjusting for dropout
n : estimated number of samples per group based on initial calculation
t : number of groups

The calculation follows Federer's formula, which aligns with WHO criteria as outlined in the Research Guidelines for Evaluating the Safety and Efficacy of Herbal Medicines, requiring a minimum of 6 rats per group. After accounting for an estimated 30% dropout rate, the adjusted sample size per group becomes 9, resulting in a total of 36 (thirty-six) rats required for the study.

The materials used in this study were as follows:

1. Thirty-six (36) male white Wistar rats (*Rattus norvegicus*) that met the inclusion and exclusion criteria.
2. *Eleutherine palmifolia* (Dayak onion) extract cream at concentrations of 10%, 15%, and 20%.
3. 70% ethanol
4. Base cream
5. Standard feed
6. 10% buffered formalin
7. Anesthetic agent (ether anaesthetic)
8. Reagents for histopathological tissue preparation:
   Bouin's solution
   10% buffered formalin
   Paraffin oil
   Albumin
   Hematoxylin and Eosin (HE) stain
   Xylol solution
   Alcohol
   Distilled water (aqua dest)

The equipment used in this study included:

1. Rat cages
2. UV light meter
3. Jar containers
4. Histopathology preparation tools (microtome and paraffin blocks)
5. Electric shaver
6. Weighing scale
7. Timer
8. Face masks and gloves
9. Blender
10. Büchner funnel
11. Centrifuge
12. UV-Vis spectrophotometer
13. Oven
14. Tools for histopathology slide preparation: coverslips, glass slides, microtome, and paraffin molds
15. 1 cc syringe

The preparation of *Eleutherine palmifolia* (Dayak onion) extract are as follows:

1. The *Eleutherine palmifolia* used in this study was sourced from Kendal region, Central Java.
2. The plant material was dried at 40°C for three days to obtain dried simplicia.
3. A total of 500 grams of powdered sample was dissolved in 1.5 liters of 70% ethanol (1:3 ratio), stirred, and allowed to stand for 20 minutes.
4. Extraction was performed using a microwave for 5–8 minutes at a power of 450 watts.
5. The extract was then filtered using Whatman filter paper to obtain the filtrate.
6. The resulting filtrate was concentrated using a rotary vacuum evaporator at approximately 100 mBar pressure, a temperature of 40–50°C, and a rotation speed of 50–60 rpm.

After that, the cream formulation process was carried out at the Cendekia Nanotech Hutama Laboratory using Synchro® base cream, which contains calendula oil, sweet almond oil, beeswax, *Hypericum* extract, fatty acid esters, vitamin B complex, vitamin A, vitamin C, vitamin E, vitamin H, biological extracts, amino acids, potassium, magnesium, glutamine, arginine, and lysine. This base was then mixed with *Eleutherine palmifolia* extract using a homogenizer to produce Dayak onion extract creams at concentrations of 10%, 15%, and 20%.

Male Wistar rats that met the inclusion criteria underwent a 7-day acclimatization period. On the eighth day, the rats were randomized. A 2x2 cm area on the dorsal region of each rat was shaved. A total of thirty-six rats were divided into four (4) groups, with each group consisting of nine rats, categorized as follows:

K: placebo cream + UVB
P1: 10% extract cream + UVB
P2: 15% extract cream + UVB
P3: 20% extract cream + UVB

UVB irradiation was administered using a pre-calibrated artificial UVB lamp placed inside a closed container to prevent the rats from escaping or turning over during exposure. The irradiation distance was set at 20 cm. UVB exposure was conducted every two days for 30 days, with each session lasting 60 minutes. In groups K, P1, P2, and P3, the cream was applied twice daily, 20 minutes before UVB exposure and 4 hours afterward.

After 30 days, all rats were terminated, and dorsal skin tissue was excised and sent to the anatomical pathology laboratory for analysis. Prior to tissue collection, the rats were anesthetized using the ether inhalation method by placing cotton soaked with 5 mL of ether at the bottom of a jar. After 5 minutes, the samples were collected with the rats positioned prone.

Epidermal thickness was assessed from skin tissue collected from male Wistar rats. The tissue was fixed in 10% buffered formalin solution for a minimum of 24 hours. Subsequently, the tissue was sagittally sectioned, placed into tissue cassettes, and labeled.

Dehydration was performed in stages using 75%, 95%, and 100% alcohol, followed by absolute alcohol I, II, and III. The tissue was then cleared using xylol I and II. The next step was embedding or paraffin infiltration using liquid paraffin I and II for 30–60 minutes in an incubator.

The processed tissue was embedded in molten paraffin, cooled for approximately 20 minutes, and sectioned using a microtome. The tissue sections were mounted on slides, dried, and stained using the Hematoxylin and Eosin (HE) method. The stained slides were then analyzed by an anatomical pathologist at the anatomical pathology laboratory.

Figure 2 illustrates the experimental workflow, including acclimatization, randomization, group treatment, UVB exposure, topical cream application, euthanasia, biopsy collection, and histopathological analysis.

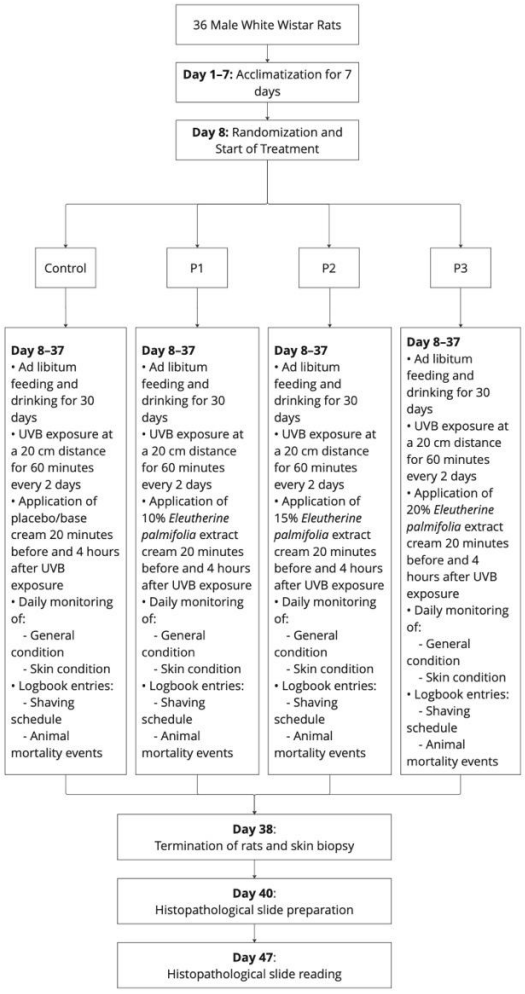

Figure 2. Research Flowchart

The data were analyzed using several statistical tests. First, the Shapiro-Wilk test was applied to assess normality, with $p \geq 0.05$ indicating a normal distribution. Homogeneity of variances was then tested using Levene's test, where $p \geq 0.05$ suggested homogeneous variances. To examine mean differences among groups, a One-Way ANOVA was conducted, followed by a Post Hoc test to determine pairwise group comparisons, with $p < 0.05$ considered statistically significant.

Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine, Universitas Diponegoro (No. 073/EC/KEPK/FK-UNDIP/III/2025). All animals were ethically treated with food and water. Procedures adhered to the approved protocol.

3. Result

A total of 36 male Wistar rats were randomly divided into four groups, each consisting of nine rats that met the inclusion and exclusion criteria. At the end of the study, animals were euthanized using ether anaesthetic. Each treatment group originally had nine rats, but only six per group were euthanized at the end of the experiment. Two rats from the control group, one from P2, and one from P3 died during the study (dropouts). No definitive cause of death was identified, and no consistent clinical signs of toxicity or treatment-related adverse effects were observed prior to death. The deaths occurred sporadically and were not associated with a specific treatment group. Final sample numbers were adjusted in line with WHO/OECD recommendations, which suggest 5–6 animals per group to maintain statistical validity. Selection of euthanized animals was randomized based on health status to ensure scientific validity.

Macroscopically, male Wistar rats exhibited erythema, seen as skin redness after treatment. This erythema is an early clinical indicator of inflammation caused by direct UVB exposure. These findings confirm that UVB radiation triggers acute inflammatory responses in skin tissue.[21,22]

Epidermal thickness was assessed using 400x magnification microscopy following HE staining. Data were tabulated and subjected to statistical analysis.

Histopathological examination was conducted to assess the effect of *Eleutherine palmifolia* cream on UVB-exposed rat skin. Epidermal thickness data were obtained using cellSens software and expressed in micrometers. Measurements were taken from five fields of view per sample, and the averages were analyzed using SPSS.

Figures 3-6 presents representative histological images from each group. The control group shows a relatively thicker epidermis due to no protection from UVB irradiation. The P1 and P2 groups demonstrate reduced epidermal thickness and improved skin structure compared to the control, while the P3 group shows the greatest reduction in epidermal thickness and the most pronounced improvement in skin architecture, indicating a dose-dependent protective effect.

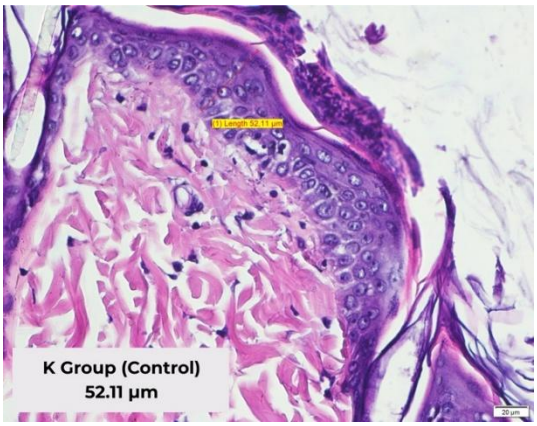

Figure 3. Representative Histological Image of Control Group

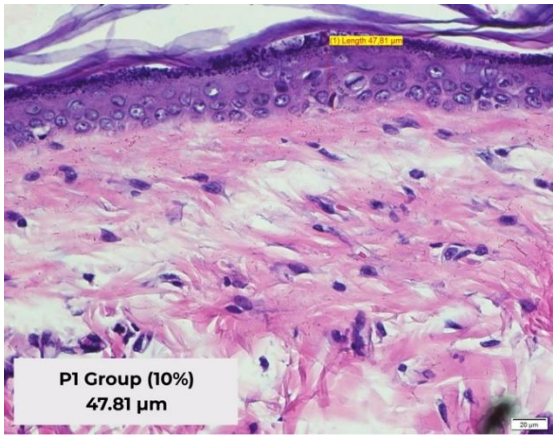

Figure 4. Representative Histological Image of P1 Group

Figure 5. Representative Histological Image of P2 Group

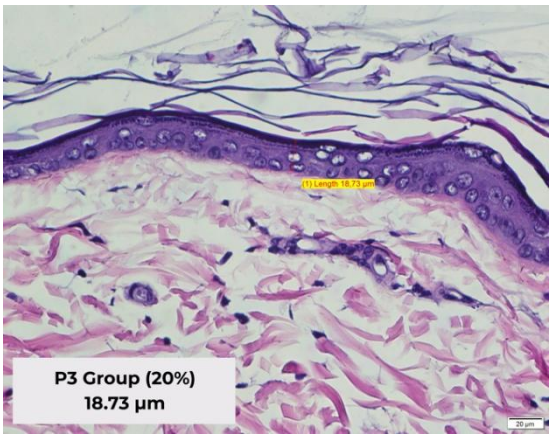

Figure 6. Representative Histological Image of P3 Group

Normality testing using the Shapiro-Wilk test yielded a p-value of 0.072 ($p > 0.05$), indicating that the data were normally distributed. This fulfills the assumption of normality, suggesting that deviations between observed and expected values are random and not systematic. This validates the use of parametric tests such as ANOVA and supports the generalizability of findings. The complete results of the Shapiro-Wilk test can be seen in Table 1.

Table 1. Shapiro-Wilk Test Results

Group	Mean ± SD (µm)	Median (min – max) (µm)	p value
Control (K)	43.52 ± 3.938	42.73 (39.19 – 51.02)	0.034
10% (P1)	32.81 ± 4.310	34.69 (26.45 – 36.56)	0.110
15% (P2)	26.07 ± 4.909	24.97 (21.40 – 35.55)	0.057
20% (P3)	22.07 ± 4.936	20.93 (16.96 – 30.99)	0.387
Residual for Epidermal Thickness			0.072

Subsequently, Levene's test for homogeneity of variances showed p-values > 0.05 across mean, median, and trimmed mean approaches, indicating homogeneous variance across groups. This supports the validity of ANOVA results and confirms that all treatment groups were evaluated under comparable conditions. The complete results of the Levene's test can be seen in Table 2.

Table 2. Levene's Test Results

Levene Statistic	p Value
Based on Mean	0.908
Based on Median	0.920
Based on Trimmed Mean	0.904

Since the data met normality and homogeneity assumptions, One-Way ANOVA was performed. The test showed significant differences in mean epidermal thickness across groups ($p < 0.001$). The complete results of the One-Way test can be seen in Table 3.

Table 3. One-Way ANOVA Results

	df	F	p Value
Between Groups	3	25.600	<0.001
Within Groups	20		
Total	23		

Post Hoc LSD analysis revealed significant differences in epidermal thickness between the control and all treatment groups ($p < 0.05$). The 10%, 15%, and 20% creams all significantly reduced epidermal thickness compared to the control. However, the difference between the 15% and 20% groups was not statistically significant ($p = 0.143$). The complete results of the LSD test can be seen in Table 4.

Table 4. LSD Post Hoc Test Results

Group	Group	Mean Difference	p value	95% Confidence Interval	
				Lower Bound	Upper Bound
Control (K)	10% (P1)	10.71	0.001	5.237	16.18
	15% (P2)	17.46	<0.001	11.98	22.93
	20% (P3)	21.45	<0.001	15.98	26.93
10% (P1)	Control (K)	-10.71	0.001	-16.18	-5.237
	15% (P2)	6.748	0.018	1.277	12.22
	20% (P3)	10.75	0.001	5.275	16.22
15% (P2)	Control (K)	-17.46	<0.001	-22.93	-11.98
	10% (P1)	-6.748	0.018	-12.22	-1.277
	20% (P3)	3.999	0.143	-1.473	9.470
20% (P3)	Control (K)	-21.45	<0.001	-26.93	-15.98
	10% (P1)	-10.75	0.001	-16.22	-5.275
	15% (P2)	-3.999	0.143	-9.470	1.473

In conclusion, all extract concentrations significantly reduced epidermal thickness compared to the control. The 20% cream had the most pronounced effect, although not significantly more than the 15% cream. Thus, 15% and 20% may offer similar benefits.

4. Discussion

This study's statistical analysis showed a significant difference in epidermal thickness among the control and treatment groups, with the One-Way ANOVA yielding a p-value < 0.001. This indicates that the differences in mean epidermal thickness across the four groups were statistically significant, supporting the hypothesis that *Eleutherine palmifolia* extract cream reduces epidermal thickness in UVB-irradiated Wistar rats. These results justified conducting a post-hoc test to determine the most significantly different groups.

The post-hoc test revealed significant differences ($p < 0.05$) between the control group and all treatment groups (10%, 15%, and 20%). Notably, the largest mean difference occurred between the control and 20% cream group (21.45 µm), suggesting that the 20% cream had the strongest epidermis-thinning effect. Conversely, the smallest mean difference was observed between the control and 10% cream group (10.7 µm), indicating a relatively weaker effect. A significant difference ($p = 0.001$) was also found between the 10% and 20% groups (mean difference = 10.74 µm), showing that higher extract concentrations enhance efficacy. However, no significant difference was observed between the 15% and 20% groups ($p = 0.143$), though the 20% cream still showed a 3.999 µm greater reduction in mean epidermal thickness. This suggests that, biologically, the 20% cream remains the most effective formulation and may serve as a benchmark for future development.

These findings align with previous research indicating that *Eleutherine palmifolia* contains potent antioxidants like flavonoids, polyphenols, and naphthoquinones (eleutherin and eleutherol), which exhibit strong anti-inflammatory and antioxidant activity.[23,24] A study by Khairiah et al. (2021) also demonstrated significant results ($p = 0.001$) using One-Way ANOVA, with the 15% concentration showing the highest efficacy, though differences with other treatment groups were not statistically significant in post-hoc analysis. This supports the ability of *Eleutherine palmifolia* cream to reduce skin inflammation, likely through its effect on keratinocyte proliferation.[25]

Similarly, Sharon et al. (2013) reported that *Eleutherine palmifolia* contains flavonoids, phenolics, and tannins with high antioxidant capacity, showing a DPPH inhibition percentage of 98.21%. These compounds help neutralize free radicals and protect the skin from UVB-induced oxidative stress, thereby contributing to reduced epidermal thickness. The present study strengthens previous findings and suggests that *Eleutherine palmifolia* cream is a promising natural topical agent for preventing epidermal thickening due to UVB exposure.[26]

Macroscopically, the appearance of erythema in the UVB-exposed Wistar rats indicates an acute inflammatory response caused by DNA and cellular damage, including the formation of cyclobutane pyrimidine dimers (CPDs).[21,22] These lesions trigger the release of inflammatory mediators such as prostaglandin E2, histamine, IL-1β, IL-6, and TNF-α through activation of signaling pathways like NF-κB, AP-1, and JAK-STAT.[27] These mediators lead to capillary vasodilation, increased vascular permeability, and immune cell infiltration (e.g., neutrophils), resulting in erythema, a common visual and biological marker of early-stage inflammation.[28,29]

UVB radiation promotes epidermal thickening via keratinocyte hyperproliferation, which is regulated by the activation of epidermal growth factor receptor (EGFR) and associated pathways such as MAPK and NF-κB.[30,31] These pathways increase the production of pro-inflammatory cytokines (TNF-α, IL-1α, IL-6), triggering rapid cell turnover and epidermal hyperplasia.[32] This serves as a protective mechanism against UVB penetration (i.e., hyperkeratosis). The application of *Eleutherine palmifolia* extract cream, rich in antioxidants, helps counter oxidative stress caused by reactive oxygen species (ROS).[33] These antioxidant compounds act through free radical scavenging, inhibition of signal transduction pathways, and suppression of MMPs and transcription factors like AP-1. As a result, collagen degradation, chronic inflammation, and excessive keratinocyte proliferation are mitigated, supporting epidermal homeostasis and reducing the risk of photoaging.[34] The low IC50 value of *Eleutherine*

palmifolia (< 50 mg/L) further confirms its potent antioxidant capacity.[35]

These mechanisms are consistent with prior research showing that topically applied antioxidants such as flavonoids, phenols, polyphenols, and naphthoquinones reduce the number of sunburn cells, suppress excessive keratinocyte apoptosis, and restore normal epidermal cell proliferation.[36,37] This biological mechanism supports the protective role of *Eleutherine palmifolia* cream in maintaining epidermal thickness near normal levels in UVB-exposed Wistar rat skin.

The lack of significant difference between the 15% and 20% groups may indicate a plateau effect in antioxidant activity. After reaching a certain threshold, further increases in concentration do not enhance biological response. This phenomenon suggests that antioxidant activity stabilizes beyond an optimal point, limiting additional benefit from higher concentrations. However, this conclusion is still based on non-significant differences and not a formal dose–response analysis, highlighting the need for further analysis.

This study has several limitations, including the absence of negative (UVB-only) and positive (gold standard) control groups. Phytochemical analysis of the base cream was not performed, and long-term safety as well as potential side effects were not assessed. The UVB exposure duration was fixed, and the effects of varying exposure times were not evaluated. Concentrations of the cream above 20% were not tested, and the study focused solely on UVB radiation without examining the effects of UVA or UVC. Additionally, clinical monitoring of skin changes was not conducted, limiting the analysis to epidermal thickness, and the stability of the cream during storage was not evaluated.

5. Conclusion

Topical *Eleutherine palmifolia* extract cream significantly reduces epidermal thickness in UVB-exposed Wistar rats. A concentration of 20% offers the greatest protection, though no significant difference was found between concentrations of 15% and 20%. These findings support *E. palmifolia* as a promising candidate for development as a natural sunscreen and anti-photoaging formulation.

Future research should include both negative (UVB-only) and positive (gold standard) control groups to strengthen comparative analysis. Phytochemical testing of the base cream should be conducted, along with evaluations of the long-term safety and effectiveness of *Eleutherine palmifolia* cream. Studies should also vary the duration of UVB exposure to assess its impact on epidermal thickness and compare different concentrations (15%, 20%, and 25%) to determine the most effective formulation. In addition, the effects of UVA and UVC exposure should be investigated, clinical skin changes and potential side effects in test animals should be monitored, and stability testing during storage should be performed to ensure consistency and sustained effectiveness. Future photoprotection studies should consider incorporating additional outcomes such as oxidative stress biomarkers (MDA, SOD, GPx), inflammatory markers (IL-6, TNF-α), and the integrity of collagen and elastin to provide a more comprehensive understanding of the skin's response to UV exposure and treatment.

Ethical Approval

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Diponegoro with Ethical Clearance Number: No.073/EC/KEPK/FKUNDIP/III/2025.

Conflicts of Interest

The authors declare no competing interests.

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Author Contributions

Conceptualization, APS and GSD; methodology, APS with input from GSD and LA; validation, APS, GSD, LA, and VK; formal analysis, APS; investigation, APS; resources, GSD and LA; data curation, APS; writing—original draft preparation, APS; writing—review and editing, GSD, LA, and VK; visualization, APS; supervision, GSD and LA; project administration, GSD; funding acquisition, GSD.

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