Jurnal Kesehatan
Vol. 15, No. 3, November 2024
ISSN 2086-7751 (*Print*), ISSN 2548-5695 (*Online*)
http://ejurnal.poltekkes-tjk.ac.id/index.php/JK

Sunscreen Effect of Andaliman Methanol Extract Nano Gel on The Amount of Melanin in Wistar Rats Exposed to UV-B Light

Vera Vera, Linda Chiuman*, Chrismis Novalinda Ginting, Atika Rimalda Nasution, Chrismas Gideon Bangun, Primta Bangun
Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia

ARTICLE INFO

Article history

Received date
13 May 2024

Revised date
24 Sep 2024

Received date
10 Oct 2024

Keywords:

Andaliman extract;
Radiation;
UV-B exposure.

ABSTRACT

Excessive exposure to UV-B rays can increase melanin production, potentially causing skin damage, making it necessary to prevent this through the use of natural-based sunscreens, such as nano gel from methanol extract of andaliman, to protect the skin from the harmful effects of UV-B radiation. This study aims to evaluate the sunscreen effect of andaliman methanol extract nanogel on the amount of melanin in Wistar rats exposed to UV-B light. The method used was a laboratory experimental study with a randomized posttest only with a control group design using mice as the research subjects. The results of the study showed that the SPF values of andaliman extract at concentrations of 2%, 3.5%, and 5% were 2.11, 2.49, and 2.45, respectively, which are considered low for sunscreen protection standards. Additionally, the amount of melanin obtained from each treatment group was also categorized as small, indicating that the andaliman extract in nano gel form did not significantly reduce melanin production in Wistar rats exposed to UV-B rays. In conclusion, there is no sunscreen effect of andaliman methanol extract nanogel on the amount of melanin in Wistar rats exposed to UV-B light. This could be due to factors such as the low SPF value, the lack of stability of the active ingredients in the nano gel, or the need for an increased dosage. The implications of these findings suggest the need for further research to optimize the formulation or explore other more effective ingredients in preventing melanin production caused by UV-B exposure.

Corresponding author:

Linda Chiuman
Faculty of Medicine, Dentistry, and Health Sciences, Universitas Prima Indonesia, Medan, Indonesia
Email: lindachiuman@unprimdn.ac.id

INTRODUCTION

Excessive exposure to UV rays has detrimental clinical signs, including erythema, pigmentation, and premature aging of the skin (IH et al., 2015; Liu et al., 2023). Ultraviolet B radiation increases the expression of several melanocyte-specific genes and stimulates the release of several factors participating in melanin synthesis (Liu et al., 2023).

It is estimated that the sun's ultraviolet radiation accounts for about 93% of skin cancers, and about half of them are lip cancers. This means there are approximately 4,500 life-threatening cancers (malignant melanoma) per year in Canada, as well as 65,000 non-life-threatening cancers (basal cell cancer, squamous cell cancer, and lip cancer). The World Health Organization (WHO) estimates that there are at least 2 million new cases of non-melanoma skin cancer and around 132,000 cases of melanoma every year (Harahap et al., 2022).

Indonesia, from Sabang to Merauke, is one of the world's centers of biodiversity. Around 40,000 plant types contain various chemicals that can be used as food ingredients, cosmetics, and medicines (Handayani et al., 2023). One area in Indonesia that has a diversity of spices from various types of plants is North Sumatra. One spice typical of North Sumatra is Andaliman (Zanthoxylum acanthopodium DC). Andaliman (Zanthoxylum acanthopodium DC) is a shrub from the Rutaceae family (citrus family) whose fruit is widely used as a traditional cooking spice by the Batak tribe and in traditional medicines such as stomach ache medicine, tonic, and antimicrobial (Ginting et al., 2023).

Phytochemical compounds in plants affect the skin and can be used as alternative cosmeceuticals for conventional medicine (Azmi, 2020; Nurlaeni & Pratiwi, 2022).

Andaliman has several biological activities, such as antioxidant, antifungal, and antibacterial, and it has potential as an antidiabetic and anticancer drug (Nurlaeni & Pratiwi, 2022). Andaliman methanol extract has also been proven to have moderate inhibitory power against one of the bacteria that causes acne, namely the Staphylococcus epidermidis bacteria (Ginting et al., 2023).

Flavonoid and polyphenolic compounds have a chemical structure similar to the substrate, causing competition between flavonoids and the substrate to enter the enzyme's active site. The phenol group in the flavonoid structure will bind to the enzyme's active site. This phenol group can also react as a copper chelator, where the phenol group will bind to the copper metal ion group (Cu2+) in the tyrosinase enzyme so that this bond can inhibit melanin production (Oktafianti et al., 2021).

Previous research also stated that phytochemical compounds have effective photoprotection activity by different mechanisms. For example, cinnamate compounds in propolis, polyphenols in tea and lichen (Lihen), and Robusta coffee leaves' phenol and flavonoid content (Yuliawati et al., 2019). The active compound Thymoquinone, which comes from black cumin, also has a strong antioxidant effect and a sunscreen effect against exposure to UVB rays (Mohamed et al., 2020). As science and technology develop, they introduce preparations in the form of nanoemulgel. Nanoemulgel is an emulsion preparation with a 1–100nm particle size suspended in a hydrogel. The smaller particle size allows better penetration and increases absorption in the skin (Chellapa et al., 2015; Imanto et al., 2019).

However, the potential of andaliman, specifically as a photoprotective agent, particularly in the form of a nanogel, has not been thoroughly investigated. Despite its known antioxidant and anti-inflammatory properties, its ability to shield the skin from UV-B-induced damage remains largely unexplored. Previous studies have focused primarily on synthetic sunscreen agents, leaving a gap in the understanding of natural alternatives like andaliman. This study aims to fill this gap by evaluating the efficacy of andaliman extract nanogel in reducing melanin production under UV-B exposure. By investigating whether andaliman can effectively mitigate melanin synthesis, which is a key factor in hyperpigmentation and UV-induced skin damage, this research seeks to contribute valuable insights into the development of natural, eco-friendly sunscreens. The findings may open new avenues for incorporating plant-based ingredients in sun protection formulations, addressing both health and environmental concerns.

The aim of this study is to evaluate the effectiveness of nano gel containing methanol extract of andaliman as a sunscreen in protecting the skin from UV-B exposure, focusing on its effect on melanin levels in Wistar rats. This research seeks to identify whether the use of andaliman extract nano gel can reduce melanin production caused by UV-B exposure, which is known as a key factor in skin hyperpigmentation and potential cellular damage. By exploring the potential of natural ingredients such as andaliman, this study aims to provide a new alternative in sunscreen formulation that is safer and more environmentally friendly.

METHOD

This study uses an experimental method by utilising a Wistar rat model that is exposed to UV-B light to simulate the effects of ultraviolet radiation on the skin. Rat models exposed to UV-B light are commonly used in scientific studies to simulate the effects of ultraviolet radiation on the skin, as they closely mimic the responses observed in human skin under similar conditions. In this model, Wistar rats are exposed to controlled doses of UV-B light over a specified period, typically simulating the damage caused by prolonged sun exposure. This UV-B exposure leads to increased melanin production, skin thickening, and potential cellular damage, making it an effective model for studying hyperpigmentation and skin protection methods. Treatment procedures often involve the application of test substances, such as sunscreens or protective agents, directly onto the skin prior to UV-B exposure. In this case, the rats are treated with varying concentrations of andaliman extract nanogel to assess its photoprotective efficacy. The melanin levels and skin condition of the treated groups are compared to control groups, which do not receive the protective treatment, allowing researchers to evaluate the effectiveness of the nanogel in preventing UV-B-induced damage.

The tools and materials used include: measuring cup, stirrer, digital balance, drinking container, rat cage measuring 36cmx28cmx12cm, microscope, glass object, cover glass, bunsen lamp, UV-B lamp, UV-Vis spectrophotometry, camera for documentation.

The andaliman fruit, known as a typical spice from Indonesia, particularly from the Sumatra and Kalimantan regions, was used in this

study as the main ingredient for the extract. The extract was prepared using distilled water and methanol, which served to extract the bioactive compounds from andaliman. In addition, Tween 80 and PEG 400 were used to produce nanoemulsion, while polyethylene glycol, carbopol, and Triethanolamine (TEA) were used in the preparation of nanogel preparations. For histological analysis, Hematoxylin Eosin (HE) Dye was used to dye tissue samples taken from the tested rats.

This research employs a true experimental laboratory design using a Randomized Post Test Only with Control Group Design to ensure robust and reliable results by eliminating potential biases from pre-testing. This design was chosen for its ability to compare the effects of treatments between groups while minimizing external influences, making it particularly suitable for examining the efficacy of andaliman extract nanogel. The study was conducted from September to November 2023 at various facilities: nanogel preparation was completed at the Pharmacology Laboratory, Faculty of Pharmacy, University of North Sumatra, and melanin histology examinations were carried out at the Pathology Anatomy Laboratory of RSU Royal Prima Medan. The method used to examine melanin histology preparations was Hematoxylin and Eosin (HE) staining, which allows for the clear visualization of cell structures, including melanocytes.

The research population consisted of 25 Wistar strain white rats (Rattus novergicus), aged 2-3 months, weighing 150-200gr, and confirmed to be healthy. The Federer formula was used to calculate the sample size, resulting in a minimum requirement of 25 rats, divided into five treatment groups, with five rats in each group. This sample size ensures that the study has sufficient statistical power to detect significant differences across the groups.

In this study, the examination of melanin histology preparations was conducted using Hematoxylin and Eosin (HE) staining. This method is commonly used in histopathology to visualize cellular structures, allowing for the identification and assessment of melanocytes in tissue samples. HE staining highlights the general morphology of the cells and tissues, making it easier to observe melanin pigment within the melanocytes.

Using HE staining allows researchers to assess the overall histological changes and the presence of melanin in the skin tissues of the rats after exposure to UV light and treatment with andaliman extract nanogel.

Research procedure

a. Making Andaliman extract nanogel

The process begins with creating a gel mass, where carbopol is developed with hot water to ensure good consistency as a thickening agent. After that, a mixture of Triethanolamine (TEA), propylene glycol, and nipagin was added and stirred until homogeneous (Ariani, 2020); TEA serves as a calcifying agent to produce a clear gel base, while propylene glycol acts as a humectant that maintains moisture (Purwandari et al., 2020).

Next, nanoemulsion of andaliman extract was added to the gel mass and mixed until homogeneous. Tween 80 was chosen as the surfactant due to its relatively safe and non-toxic nature, which is important for maintaining the safety of the formulation, especially for use on the skin. Polyethylene glycol (PEG 400) was used as a co-surfactant and humectant, thanks to its good solubility in water, which helps to improve the stability and effectiveness of the gel. The selection of these ingredients was critical to achieving the research objective, which was to develop a nano gel formulation that is effective in providing protection against UV-B exposure, while ensuring user safety and convenience.

Table 1. Formulation of andaliman extract nano gel

Material (%)	Formula I	Formula II	Formula III
Nanoemulsion	2	3.5	5
Carbophol	1	1	1
TEA	2	2	2
Propylene glycol	5	5	5
Methylparaben	0.5	0.5	0.5
Aquadest arrived	100	100	100

b. Treatment of experimental animals

This study has adhered to ethical approval for the use of animals, which is an important step in ensuring that all research procedures are conducted with serious consideration for animal welfare. Before commencing the experiments, our research protocol was submitted and evaluated by the relevant ethics committee, which ensured that all actions taken were in accordance with established guidelines to avoid unnecessary suffering for the animals. We are committed to upholding the highest standards in research ethics, so that the results obtained are not only scientifically valid but also morally responsible.

The methanol extract of andaliman in the nano gel preparation was divided into three concentrations. The initial research began by

preparing 25 male Wistar rats acclimatized for seven days. Experimental animals were grouped randomly into five groups of five animals each. Animals were housed in individual cages that were cleaned daily. Mice were given standard food and drinking water ad libitum throughout the study.

Based on previous research, skin darkening results from continuous exposure to sunlight for a maximum of approximately three weeks ((Layuck et al., 2015; Mohamed et al., 2020). In this study, exposure to UV-B rays was carried out using an Exoterra UVB 200 13-watt lamp for +- 2 hours at a distance of 30 cm from the skin every day (Sari et al., 2020). The research design is a Randomized Post Test Only with a Control Group Design with group design:

- Group A negative control mice
- Group B mice applied SPF 15 sunscreen 30 minutes before being exposed to UV light for 21 days
- Group C mice were smeared with andaliman F1 nano gel 30 minutes before being exposed to UV light for 21 days
- Group D mice were smeared with andaliman F2 nano gel 30 minutes before being exposed to UV light for 21 days
- Group E mice were treated with Andaliman F3 nano gel for 30 minutes before exposure to UV light for 21 days.

The rat's back was shaved to an area of 4x4cm. Andaliman nano gel is given with a brush applied to the back 4x4 cm. After the final irradiation, all mice from the five groups were euthanized, their back skin tissue was taken, and placed in a 40% formalin solution. Histological preparations were made of mouse skin tissue to examine the amount of melanin.

c. Melanin examination

The skin organs of control and treated mice were processed to create histopathological preparations and evaluated for the amount of skin melanin pigment using Hematoxylin Eosin (HE) staining. HE staining is the preferred method for melanin evaluation due to its effectiveness in highlighting cellular structures and differentiating various tissue components. This staining technique allows for clear visualization of melanin deposits within the skin tissue, enabling accurate assessment of melanin levels. The amount of melanin pigment calculated is determined by the visible melanin pigment per one field of view under microscopic magnification of 100x10, with the interpretation categorized into three groups: little (<40 melanin pigments), moderate (40-80 melanin pigments), and a lot (>80 melanin pigments). By using HE staining, researchers can obtain reliable data on melanin distribution and density, which is crucial for understanding the effects of treatments on skin pigmentation

d. Examination of the SPF value of Andaliman extract nanogel

Determination of the SPF value was carried out using a UV-Vis spectrophotometer by weighing 0.5gr of andaliman extract nano gel with concentrations of 2%, 3.5%, and 5%, respectively. The extract was then dissolved in 25mL of methanol, ultrasonicated for 5 minutes, and filtered through filter paper before being read in a UV-Vis spectrophotometer at a UVB light wavelength of 290-320 nm at intervals of every 5 nm. The test was conducted three times, and the SPF value was calculated by inputting the absorbance data into the Mansur equation.

The Mansur equation is particularly suitable for this study as it provides a reliable means of calculating SPF based on the absorbance of UV radiation, which directly correlates to the protective efficacy of the formulation. This method takes into account the amount of light absorbed by the sunscreen, allowing for an accurate estimation of its protective capabilities against UVB exposure. However, there are potential limitations to this method. For instance, the Mansur equation assumes uniform application of the product and does not account for factors such as skin type, application thickness, or environmental conditions, which may affect real-world SPF performance. Additionally, the use of a laboratory setting may not fully replicate the complex interactions that occur during actual sun exposure, highlighting the need for further in vivo testing to validate these findings. Determination of the SPF value is carried out based on the Mansur equation, which is as follows:

$$\mathrm{SPF}_{in\ vitro} = \mathrm{CF} \times \sum_{290}^{320} \mathrm{EE}(\lambda) \times I(\lambda) \times \mathrm{abs}(\lambda)$$

CF is the correction factor (=10), EE is the erythema effect spectrum, I is the light intensity spectrum, and Abs is the sample absorbance. The SPF value ranges from 0 to 100; according to the FDA, sunscreen ability is considered reasonable above 15 (Tamara et al., 2020). The EE x I value is a determination, as seen in Table 2 below (Yuliawati et al., 2019).

Table 2. EE x I Values

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1

e. Evaluation of Andaliman Methanol Extract Nanogel Preparations

- Homogeneity Test

  Homogeneity checking was carried out using a glass object. This test was carried out using two glass objects. The homogeneity of the preparation is checked by smearing it on a glass object, leveling it with another glass object, and observing it. Observations are made by examining whether particles have yet to be mixed homogeneously. Achieving homogeneity is crucial, as it ensures consistent formulation, which directly affects the product's efficacy and stability.

- Test pH

  The pH test was carried out by dipping the pH meter into the andaliman extract nanogel preparation. One gram of the preparation was dissolved in 10ml of water, and then the pH was measured using a pH meter. The ideal pH range for the cream, aligning with skin pH (4.2-6.5), is important for maintaining skin health and preventing irritation. A formulation within this range enhances skin compatibility, promoting better consumer acceptance and efficacy.

- Organoleptic Test

  This test involved observing changes in the shape, color, and odor of the nanogel preparation. The organoleptic evaluation aims to gauge consumer preference regarding the formulation's aesthetic and sensory properties. Positive sensory attributes can significantly influence user satisfaction and adherence to product use, impacting its overall acceptance in the market.

- Spreadability Test

  Spreadability was measured by measuring the diameter of a sample placed around 1g between two horizontal glasses (10x10cm) after adding a load of 125g to the top of the disk for 1 minute. Then, measure vertically and horizontally using a ruler. The average value of both is determined as the dispersive power diameter. A good spreadability range of 5 to 7cm is essential for effective application, as it facilitates extensive contact between the nanogel and the skin, leading to quicker absorption of the active ingredients. This characteristic is vital for consumer satisfaction, as it enhances the overall user experience (Lamusu, 2018).

Data analysis

The data analysis included a normality test utilizing the Kolmogorov-Smirnov test to assess whether the data followed a normal distribution. If the data were found to be normally distributed and homogeneous, a One Way ANOVA test was performed to evaluate the significance among the different groups. This test is particularly relevant as it allows for comparison of means across multiple groups, directly addressing the research objective of assessing the efficacy of andaliman extract nanogel at varying concentrations. Conversely, if the data were not homogeneous, the Kruskal-Wallis test was employed as a non-parametric alternative. This test is appropriate for analyzing ranked data and offers insights when the assumptions of ANOVA are violated. Statistical tests were conducted at a confidence level of 95% ($p<0.05$), ensuring that the findings are statistically significant and robust, thereby reinforcing the validity of the research conclusions.

RESULTS

Table 3. Screening results phytochemicals

Secondary Metabolites	Results
Flavonoids	+
Alkaloids	+
Saponin	+
Glycosides	+

The table 3 presents the results of the phytochemical analysis of andaliman methanol extract, which reveals the presence of several secondary metabolites, including flavonoids, alkaloids, saponins, and glycosides. Each of these phytochemicals plays a significant role in enhancing the antioxidant properties of the nanogel. For instance, flavonoids are known for their potent antioxidant activity, helping to scavenge free radicals and reduce oxidative stress, which is crucial in protecting skin cells from UV damage. The addition of 5% FeCl3 to the andaliman methanol extract results in a color change; a darker color indicates a positive presence of phenolic compounds, contributing to

the extract's antioxidant capabilities (Ikalinus et al., 2015). Similarly, the stability of foam formation upon adding one drop of 2N HCl indicates the presence of saponins, which can enhance the emulsifying properties of the nanogel, further promoting its effectiveness in delivering active compounds (Novianti et al., 2019).

Table 4. Organoleptic evaluation test

Organoleptic evaluation	F1	F2	F3
Aroma	Distinctive smell	Distinctive smell	Distinctive smell
Color	Chocolate	Chocolate	Chocolate
Texture	Gel	Gel	Gel

The organoleptic test results of the andaliman methanol extract nanogel in each formulation reveal a distinctive andaliman aroma, a brownish color, and a gel-like texture. These organoleptic properties play a crucial role in user acceptance; for instance, a pleasant and recognizable aroma can enhance the overall sensory experience, making users more likely to incorporate the product into their skincare routine. Additionally, the brownish color, while characteristic of the andaliman extract, could influence perceptions of naturalness and quality. The gel-like texture contributes to the product's application, allowing for smooth spreading on the skin, which can enhance user satisfaction. Moreover, the stability of these organoleptic properties is vital for maintaining the product's appeal over time. If the aroma or color changes adversely during storage, it could impact both the efficacy and acceptance of the nanogel, emphasizing the importance of formulating a stable product that retains these desirable characteristics.

Table 5. Homogeneity and pH Test

Formulation	Homogeneity Test	Test pH
F1	Homogeneous	6.42
F2	Homogeneous	6.38
F3	Homogeneous	6.36

The table above shows the results of the homogeneity test and pH test for each formulation of the andaliman extract nanogel preparation. It was found that the nanogel preparation observed was mixed homogeneously. The pH test showed that the pH of the andaliman extract nanogel was in the range of 6.36 - 6.42, where the higher the concentration of andaliman extract, the lower the pH. The pH results of the andaliman extract nanogel correspond to the skin pH of 4.2-6.5 (Lamusu, 2018).

Maintaining a pH within this range is crucial for skin applications, as it helps preserve the skin's natural barrier function, minimizing irritation and promoting better compatibility. In comparison to similar products that utilize different botanical extracts, such as aloe vera (Maan, 2018) and tea tree oil (Yadav, 2017), this pH balance may enhance user acceptance and reduce adverse reactions. Furthermore, formulations that stray too far from this optimal range can compromise skin integrity, reinforcing the significance of carefully selecting and adjusting the formulation's pH to ensure both efficacy and safety for the user. By aligning with findings from earlier research, this study underscores the importance of pH in the formulation of skin applications.

Table 6. Spreadability test

Formulation	F1			F2			F3		
Load (g)	0	100	125	0	100	125	0	100	125
Spread Power (cm)	3.5	4	4.4	4	4.5	4.8	4.2	4.7	4.9

The table 6 shows the results of the spreadability test of andaliman extract nanogel using loads of 0, 100, and 125 g. It was found that the F1 spreadability range was 3.5 to 4.4 cm. F2 has a spread power range of 4 to 4.8 cm, and F3 has a spread power of 4.2 to 4.9 cm. The results obtained do not comply with the criteria for good spreadability of cream, namely 5 to 7 cm (Lamusu, 2018).

To improve spreadability, modifications in the formulation could be considered. For example, increasing the proportion of humectants or emollients, such as glycerin or propylene glycol, may enhance the product's fluidity, making it easier to apply. Additionally, adjusting the concentration of the gelling agent (carbopol) could help achieve a balance between viscosity and spreadability. These adjustments would not only improve the ease of application but also enhance the product's overall usability, potentially increasing consumer satisfaction by providing a smoother and more even coverage on the skin. Ensuring better spreadability could also lead to more efficient absorption of active ingredients, thus enhancing the nanogel's effectiveness.

Table 7. SPF value check results

Wavelength (nm)	EE x I	EE x I x Abs		
		Extract 2%	Extract 3.5%	Extract 5%
290	0.0150	0.006735	0.007935	0.00819
295	0.0817	0.0241015	0.0288401	0.029412
300	0.2874	0.0686886	0.0807594	0.0796098
305	0.3278	0.0662156	0.0767052	0.0744106
310	0.1864	0.0313152	0.0370936	0.0359752
315	0.0839	0.0120816	0.0147664	0.0144308
320	0.0180	0.002268	0.002862	0.002826
Total	1	0.2114055	0.2489617	0.2448544
SPF		2.114055	2.489617	2.448544

From Table 7, the SPF value obtained is based on the Mansur equation from the absorbance value obtained using UV-Vis and CF spectrophotometry, which is 10. So, based on the calculation results above, 2% andaliman extract nanogel has an SPF of 2.11, 3.5% andaliman extract has an SPF of 2.49, and 5% andaliman extract has an SPF of 2.45.

Based on the assessment of the Food and Drug Administration (FDA), the 2%, 3.5%, and 5% andaliman extract nanogel protection types are the minimum protection types because they are in the SPF 1-4 range (Lisnawati et al., 2019). The SPF 2 calculation results on andaliman extract nano gel indicate that andaliman extract nano gel protects the nano gel for 20 minutes against UV rays.

To enhance the SPF of the andaliman extract nanogel, several modifications could be considered. For instance, incorporating natural or synthetic UV filters such as zinc oxide or titanium dioxide could significantly boost the sun protection factor. Additionally, increasing the concentration of flavonoids or other UV-absorbing phytochemicals in the andaliman extract may improve its photoprotective properties. Furthermore, combining the extract with antioxidants like vitamin E or C could offer synergistic effects, enhancing both SPF and overall skin protection against UV-induced damage. These modifications would help optimize the nanogel's efficacy as a sunscreen and potentially increase its market appeal.

Table 8. Average melanin amount

Treatment Group	Melanin Amount	Melanin Category
Control	< 10	A little
Positive contrast	< 10	A little
Andaliman 2%	< 10	A little
Andaliman 3.5%	< 10	A little
Andaliman 5%	< 10	A little

The data was processed using the Shapiro-Wilk normality test to obtain a significance of 0.013 (<0.05), which means the data is not normally distributed. The test was then continued with the Kruskal Wallis test, and Asymp results were obtained. Sig. 0.406 (>0.05), so it is stated that there is no significant relationship between andaliman and the amount of melanin between the research groups.

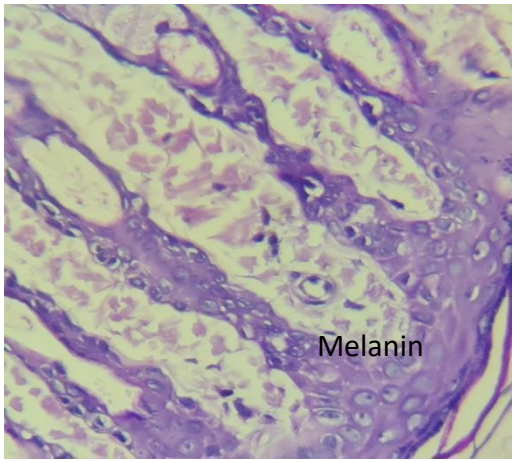

Figure 1. Photo results of melanin examination with HE (Haematoxylin-Eosin) staining

Figure 1 is a histological section stained with Hematoxylin and Eosin (HE) showing the presence of melanin in the skin tissue. Melanin, which appears as areas of darker pigmentation, is primarily located in the lower layer of the epidermis, likely within the melanocytes found in the stratum basale. HE staining allows for a clear differentiation of tissue structures, where the lighter areas represent less pigmented cells, and the darker regions correspond to areas containing melanin. This indicates that melanin is produced in response to UV exposure, serving as a natural protective barrier against UV radiation by absorbing and dispersing it, thereby preventing DNA damage in the deeper layers of the skin. Such histological analysis can provide insights

into the effectiveness of andaliman extract nanogel in influencing melanin production and skin protection.

Examining the amount of melanin showed that each treatment group had a total of less than ten melanin. The amount of melanin pigment was counted per field of view and at a microscopic magnification of 100x10. The amount of melanin obtained was small because there were <40 melanin pigments (Yonathan et al., 2016). Based on the data obtained, it is known that andaliman methanol extract nano gel does not have a sunscreen effect in inhibiting melanin formation in mice exposed to UV light. This can occur due to several causal factors, such as the staining method, exposure time, and research subjects used in this study.

The lack of effect could be attributed to several factors, including the specific interaction of andaliman with melanin synthesis pathways. Andaliman may not have a strong inhibitory effect on the key enzymes involved in melanin production, such as tyrosinase, which plays a critical role in the melanogenesis process. Additionally, the exposure time to UV light, the concentration of active compounds in the nanogel, or the formulation's ability to penetrate the skin may not have been sufficient to produce a measurable impact on melanin production.

To enhance the formulation's effectiveness, alternative strategies could include increasing the concentration of andaliman extract or combining it with other known melanin-inhibiting agents such as niacinamide, kojic acid, or arbutin. Furthermore, modifying the delivery system to improve skin penetration, or extending the duration of UV exposure in future studies, could help to better evaluate the potential sunscreen and melanin-inhibiting effects of the nanogel.

DISCUSSION

Testing the effectiveness of andaliman methanol extract nano gel as a sunscreen on the amount of melanin has never been carried out before, however testing the efficacy of andaliman extract as anti-aging and anti-acne has been carried out previously by (Nurlaeni & Pratiwi, 2022). This research shows that andaliman extract gel can moisturize, and reduce black spots and wrinkles. The flavonoid content is known to increase the amount of collagen by inhibiting the work of matrix metalloproteinase so that it can increase the amount of collagen in the skin layer and inhibit the decrease in collagen levels after exposure to UV light (Sari et al., 2020). The flavonoid content in andaliman extract can also prevent skin damage due to exposure to UV rays, thereby reducing skin aging. Flavonoids have a role as inhibitors of the tyrosinase enzyme so that they can inhibit tyrosine from becoming DOPA and Dopaquinone so that melanin formation is inhibited (Hanum & Laila, 2018).

In this study, sunscreen with SPF 15 was used as a positive control, while only a gel base was given for the negative control. In both control groups, the amount of melanin was less than ten and was in the low category. In another study, mice exposed to sunlight for 20 days experienced a significant increase in the amount of melanin compared to the negative control group, so it can be concluded that exposure to sunlight increases the amount of melanin pigment (IH et al., 2015). Research by (Yonathan et al., 2016) also showed increased melanin in Wistar rats exposed to UV light compared to the control group.

This research uses conventional Hematoxylin Eosin (HE) staining to see melanocytes in tissue. In conventional staining methods, melanocytes only appear incidentally as clear cells in the stratum basalis. In Layuck et al.'s research (2015), the staining used to see the amount of melanin in mice was the Fontana Masson staining. The image of melanin pigment will appear as blackish-brown grains of melanin pigment.

Based on the results obtained in this study, the 5% andaliman extract nanogel exhibited the highest potential for melanin inhibition, although the overall melanin count remained below the threshold for significant sunscreen activity. Compared to other concentrations, such as 2% and 3.5%, the 5% concentration did not show a substantial improvement in melanin reduction, suggesting that the extract's efficacy plateaus at higher concentrations.

In comparison to Layuck et al.'s study, where the use of Fontana Masson staining provided more distinct insights into melanin content, the current study's reliance on conventional HE staining may have contributed to underestimating the true effect of andaliman extract on melanin formation. This suggests that future research may benefit from adopting more specialized staining techniques to better evaluate the melanin-inhibiting properties of higher andaliman concentrations or combining it with other melanin-suppressing agents for improved efficacy.

This research demonstrated that the use of andaliman methanol extract nanogel did not yield significant effects on the reduction of melanin in white mice exposed to UV light. Several factors

may explain this outcome, including the staining method employed, which may have hindered accurate observation of melanocytes. Specifically, conventional Hematoxylin Eosin (HE) staining reveals melanocytes as clear cells, making them more difficult to detect. Additionally, the research subjects had minimal melanin pigmentation, which could have further complicated efforts to observe significant changes (Yonathan et al., 2016).

Limitations of this study include the use of a single method to assess SPF and melanin reduction, as well as the short duration of UV exposure. These factors may have affected the effectiveness of andaliman extract nanogels in providing maximum protection against UV light and inhibition of melanin formation.

Future research could explore alternative methods to address these limitations. Using more sensitive staining techniques, such as Fontana Masson, could provide clearer insights into melanin changes. Furthermore, reformulating the nanogel to include additional active ingredients, such as more potent UV filters or antioxidants, may enhance its efficacy. A deeper investigation into the mechanisms of melanin synthesis and how andaliman extract interacts with these pathways could also guide improvements in the formulation.

CONCLUSION

The objective of this research was to evaluate the effectiveness of andaliman methanol extract nanogel as a sunscreen and its impact on melanin production in mice exposed to UV-B light. The primary findings reveal that the andaliman methanol extract nanogel has an SPF of 2, placing it in the minimal protection category. Additionally, the nanogel did not demonstrate a significant effect on reducing melanin levels in the tested mice. These results suggest that, although the nanogel offers some level of sun protection, it is not sufficiently effective as a sunscreen in its current formulation. The findings highlight the need for further optimization of the formulation, possibly through combining the extract with other photoprotective agents, or adjusting its concentration and application method.

Future research should explore alternative formulations of the nanogel, extend the duration of UV exposure, and utilize diverse methods for assessing SPF and melanin reduction. This would help to better understand the extract's potential and address the limitations observed in this study. In conclusion, while andaliman methanol extract nanogel presents some promise for sun protection, additional research and formulation improvements are essential to enhance its efficacy as both a sunscreen and a melanin-reducing agent.

REFERENCES

Ariani, L. W. (2020). Formulasi Dan Stabilitas Fisik Sediaan Nanogel Minyak Biji Matahari. *REPOSITORY STIFAR*.

Azmi, A. S. (2020). Kosmeseutikal Herbal Pada Hiperpigmentasi Pascainflamasi (Hpi). *Gema Kesehatan*, *12*(2), 44–56.

Chellapa, P. et al. (2015). Nanoemulsion and Nanoemulgel as a Topical Formulation. *IOSR J. Pharm. 5*(10): 43–7. 6.

Ginting, L. E. B., Kotsasi, F., Chiuman, L., Nadapdap, T., Liena, L., & Pardianto, G. (2023). Efektivitas antimikroba ekstrak buah andaliman terhadap bakteri Klebsiella pneumoniae. *Buletin Kedokteran & Kesehatan Prima*, *2*(2), 27-31. https://jurnal.unprimdn.ac.id/index.php/bulkesprima/article/view/4701

Handayani, L., Septiani, G., Putra, D. A. W., Aina, N., Darweni, S., & Rio, A. (2023). An Exploration of Phytochemical Content in The Endemic Flora of Seruyan District, Central Kalimantan. *JST (Jurnal Sains Dan Teknologi)*, *12*(3). https://doi.org/10.23887/jstundiksha.v12i3.68062

Hanum, T. I., & Laila, L. (2018). Evaluation of the anti-aging and anti-acne effect of Andaliman (Zanthoxylum acanthopodium DC.) ethanolic extract peel-off gel mask. *Asian Journal of Pharmaceutical and Clinical Research*, *11*(13), 90.

Harahap, E. S., Ginting, C. N., Lubis, Y. M., & Chiuman, L. (2022). The Activity of Strawberry Extract as Sunscreen on Guinea Pigs Exposed to Sunlight. *IOP Conference Series: Earth and Environmental Science*, *1083*(1), 012007. https://doi.org/10.1088/1755-1315/1083/1/012007

IH, S. F., Lintong, P. M., & Loho, L. L. (2015). Pengaruh pemberian umbi bengkuang (Pachyrrhizus erosus l urban) terhadap jumlah pigmen melanin kulit mencit (Mus musculus) yang dipaparkan sinar matahari. *EBiomedik*, *3*(1). https://doi.org/10.35790/ebm.v3i1.6641

Ikalinus, R., Widyastuti, S. K., & Setiasih, N. L. E. (2015). Skrining fitokimia ekstrak etanol kulit batang kelor (Moringa oleifera). *Indonesia Medicus Veterinus*, *4*(1), 71–79.

Imanto, T., Prasetiawan, R., & Wikantyasning, E. R. (2019). Formulasi dan karakterisasi sediaan nanoemulgel serbuk lidah buaya (Aloe vera L.). *Pharmacon: Jurnal Farmasi Indonesia*, *16*(1), 28-37. https://journals.ums.ac.id/index.php/pharmacon/article/view/8114

Lamusu, D. (2018). Uji organoleptik jalangkote ubi jalar ungu (ipomoea batatas l) sebagai upaya diversifikasi pangan. *Jurnal Pengolahan Pangan*, *3*(1), 9–15. https://doi.org/10.31970/pangan.v3i1.7

Layuck, A. R. P., Lintong, P. M., & Loho, L. L. (2015). Pengaruh pemberian air perasan jeruk nipis (Citrus aurantifolia) terhadap jumlah pigmen melanin kulit mencit (Mus musculus) yang dipaparkan sinar matahari. *EBiomedik*, *3*(1). https://doi.org/10.35790/ebm.v3i1.6613

Lisnawati, N., NU, M. F., & Nurlitasari, D. (2019). Penentuan Nilai Spf Ekstrak Etil Asetat Daun Mangga Gedong Menggunakan Spektrofotometri Uv-Vis. *Jurnal Riset Kefarmasian Indonesia*, *1*(2), 157–165. https://doi.org/10.33759/jrki.v1i2.35

Liu, W., Chen, Q., & Xia, Y. (2023). New mechanistic insights of melasma. *Clinical, Cosmetic and Investigational Dermatology*, pp. 429–442. https://www.tandfonline.com/doi/full/10.2147/CCID.S396272

Maan, A. A., Nazir, A., Khan, M. K. I., Ahmad, T., Zia, R., Murid, M., & Abrar, M. (2018). The therapeutic properties and applications of Aloe vera: A review. *Journal of Herbal Medicine*, *12*, 1-10. https://doi.org/10.1016/j.hermed.2018.01.002

Mohamed, M. B. M. (2020). Oily in situ gels as an alternative floating platform for ketoconazole release. *International Journal of Research in Pharmaceutical Sciences*, *11*(2), 2638-2649. https://doi.org/10.26452/ijrps.v11i2.2278

Novianti, T., Zainuri, M., & Widowati, I. (2019). Aktivitas antioksidan dan identifikasi golongan senyawa aktif ekstrak kasar mikroalga chlorella vulgaris yang dikultivasi berdasarkan sumber cahaya yang berbeda. *Barakuda'45*, *1*(2), 72–87. https://doi.org/10.47685/barakuda45.v1i2.44

Nurlaeni, Y., & Pratiwi, R. A. (2022a). Andaliman (Zanthoxylum Acanthopodium Dc.) Tumbuhan Rempah Sumatera Utara: Pemanfaatan Dan Potensi. *Prosiding Seminar Nasional*, 345–356.

Oktafianti, R., Soewondo, B. P., & Aryani, R. (2021). Kajian Pustaka Pemutih Kulit dari Bahan Alam serta Formulasinya dalam Sediaan Nanoemulsi. *Prosiding Farmasi*, 573–578.

Purwandari, V., Sianipar, A. Y., Silalahi, Y. C. E., & Nasution, D. J. (2020). Uji Efektivitas Antibakteri Nano Gel Bahan Aktif Ekstrak Kayu Manis (Cinnamomum burmannii) Terhadap Staphylococcus aureus. *Jurnal Farmanesia*, *7*(2), 92–99. https://doi.org/10.51544/jf.v7i2.2776

Sari, W., Chiuman, L., Ginting, S. F., & Ginting, C. N. (2020). Pengaruh Krim Ekstrak Jintan Hitam (Nigella Sativa) Terhadap Kadar Kolagen Dan Hidrasi Kulit Pada Tikus (Rattus Norvegicus) Galur Wistar Jantan Yang Dipapar Sinar Ultraviolet-B. *Berita Biologi*, *19*(3A), 321–325. https://doi.org/10.14203/beritabiologi.v19i3A.3863

Tamara, A., Harjanti, R., & Nilawati, A. (2020). Evaluasi Aktivitas Tabir Surya Krim Ekstrak Etanol Buah Tomat (Solanum lycopersicum L.) Secara in Vitro dan in Vivo. *Prosiding Seminar Nasional Unimus*.

Yadav, E., Kumar, S., Mahant, S., Khatkar, S., & Rao, R. (2017). Tea tree oil: a promising essential oil. *Journal of Essential Oil Research*, *29*(3), 201-213. https://doi.org/10.1080/10412905.2016.1232665

Yonathan, K. H., Lintong, P. M., & Durry, M. F. (2016). Pengaruh pemberian ekstrak biji kakao (Theobroma cacao) terhadap jumlah pigmen melanin kulit tikus Wistar (Rattus novergicus) yang dipapar sinar matahari. *EBiomedik*, *4*(2). https://doi.org/10.35790/ebm.v4i2.14710

Yuliawati, K. M., Sadiyah, E. R., Solehati, R., & Elgiawan, A. (2019). Sunscreen activity testing of robusta coffee (Coffea cenephora ex Froehner) leaves extract and fractions. *Indonesian Journal of Pharmaceutical Science and Technology*, *1*(1), 24–29.