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Preclinical Study of Hibiscus Leaves (*Hibiscus rosa-sinensis* L.) as Simplicial Ointment on Albino Rats (*Rattus norvegicus*)

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ABSTRACT

The growing threat of skin damage due to ultraviolet (UV) exposure, especially in tropical climates such as Indonesia, has encouraged the search for innovative solutions to prevent skin damage. The hibiscus plant (*Hibiscus rosa-sinensis* L.), which is abundant in Indonesia, offers exciting prospects as a source of photoprotective compounds and antioxidants for dermatological applications. The objective of this work was to preliminarily evaluate the potential use of hibiscus leaf simplicia ointment in protecting the skin from ultraviolet light-induced damage and to assess its acute dermal toxicity. The activity and acute dermal toxicity test were conducted on white albino rats (*Rattus norvegicus*) maintained under UV light conditions as a research model. An acute dermal toxicity test was performed in accordance with the Organization for Economic Co-operation and Development (OECD) Guideline 402 fixed dose procedure. Hair growth, skin histology, and blood profiles were examined by activity test. According to the findings, the use of hibiscus leaf simplicia ointment kept the red blood cell count, hematocrit value, hemoglobin level, and total leukocyte count close to normal in rats exposed to UV light. Moreover, the preparation was capable of stimulating hair growth, with the 40% concentration group showing better hair growth than the positive and negative controls on days 15 and 18, respectively. Nonetheless, histological examination suggested that the fabrication did not allow for the maintenance of normal skin in the ultraviolet-exposed rats. Acute dermal toxicity tests resulted in LD50 values > 2000mg/kg BW, indicating that the ointment was non-irritating. Hence, hibiscus leaf simplicia ointment could serve as a UV-protective agent and a hair growth promoter. Additional studies on the mechanism of histopathological skin protection and the design of optimal ointments are required.

Keywords: Acute dermal toxicity, Hair growth, Hibiscus leaf, Simplicia ointment, Ultraviolet radiations.

INTRODUCTION

Indonesia is a country situated on the equator, resulting in a tropical climate that receives abundant sunlight throughout the year (Aldrian et al. 2022). As a result of getting abundant sunlight, it can increase skin damage due to ultraviolet (UV) rays (Farris and Valachhi 2022). One of the oxidative effects of sunlight is the presence of ultraviolet (UV) rays, which induce skin damage through an inflammatory mechanism when exposure is prolonged (Lee et al. 2023; Verma et al. 2023). UV radiation is divided into three categories: UVC, UVB, and UVA, with

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wavelengths of 100-280nm, for UVC, 290–320nm for UVB, and 320–400 nm for UVA, respectively (Ayad et al. 2023). These harmful effects not only impact humans but also animals, especially those whose habitats are located in areas with high temperatures or in tropical regions (Tang et al. 2024). This condition most frequently affects animals with epidermal pigment deficiency, sparse hair, hair loss, or those at high risk of skin diseases (Giannone et al. 2023). The harmful effects of UV rays can be minimized by using ingredients with UV-protective properties, such as antioxidant compounds (Pratiwi and Husni 2017). Antioxidants can be obtained from natural and synthetic compounds (Kabous et al. 2025). Many plants studied act as antidotes to UV rays, one of which is hibiscus (Al-Snafi 2018).

Hibiscus (*Hibiscus rosa-sinensis L.*), also known as "Pucuk Bang" among the Balinese, is an ornamental plant that is quite popular among Indonesian people (Iriani et al. 2020). Every part of the hibiscus plant contains phytochemicals that have various benefits. Hibiscus leaves contain flavonoids, polyphenols, calcium oxalate, peroxidase, tannins, terpenoids, and saponins (Kowti et al. 2020; Kapoor et al. 2021). Apart from that, ß-sitosterol, teraxeryl acetate, and malvalic acid can be found only in leaves and stems (Missoum 2018). Hibiscus leaves can be used to treat androgenic alopecia (Chakraborty et al. 2023). They can improve kidney function (Ajiboye et al. 2024), reduce stomach acidity, and treat peptic ulcers (Rajesham et al. 2024). Hibiscus leaves are used in the treatment of fever and as cough medicine (Amtaghri et al. 2024). They also play a role in wound healing (Rambe et al. 2022), have hypoglycemic activity, and natural insulinotropic effects. They also possess anti-inflammatory properties and stimulate hair growth. They are analgesic, antipyretic, antibacterial, and antioxidant (Al-Snafi 2018).

There has been a huge demand for herbal medicines and complementary and alternative therapies (CAMs) from the public for the prevention and treatment of diseases, especially during the COVID-19 pandemic in 2019 (Paudyal et al. 2022). Traditional medicine usage in Indonesia is based on the degree of knowledge about these medicinal practices. Currently, the main problems are a limited understanding of medicinal ingredients and the spread of misinformation about the effectiveness of traditional medicine (Amali et al. 2023; Barvaliya et al. 2023). The concept that the use of traditional medicines is a major cause of the community's belief that these medicines are naturally safer, have fewer side effects, and may easily be obtained from pharmacies without prescriptions still allows these misconceptions to exist (Agrawal et al. 2024). In line with synthetic pharmaceuticals, traditional medicines are also disclosed to have both intrinsic and extrinsic toxicological risks (Jităreanu et al. 2022). For example, it has been reported that saponin and alkaloid components in hibiscus flowers are one of the causes of skin irritation (Wang et al. 2022; Amtaghri et al. 2023). This is one of the main reasons why preclinical testing plays an important role in determining the safety and effectiveness of medicinal ingredients (Mugale et al. 2024).

The most crucial connection in drug development is that between drug discovery and clinical trials, mediated by pre-clinical studies. And this is a critical step that must be done for drugs to get into the hands of patients. The safety, effectiveness and pharmacokinetics of candidate drugs are tested at this point by *in vitro* and *in vivo* means (Negi et al. 2023). The test will provide information on the pharmacological effect, pharmacokinetic profile, and toxicity of the drugs through scientific tests such as toxicity and activity tests (Gambacurta et al. 2024; Scotti and Scotti 2025). This study was prepared for immediate toxicity investigations, acute dermal toxicity, and activity reactions tests that would be carried out to evaluate the effect or influence of this medication on hair growth, skin profile, and blood picture of white rats (*Rattus norvegicus*).

MATERIALS AND METHODS

Animals and management

For the acute dermal toxicity test, adult female albino rats (*Rattus norvegicus*) weighing 200–300g, nulliparous, and in good physical condition between the ages of 12 and 16 weeks were employed. The OECD Guideline 402 (OECD 2017) served as the basis for the number of animals used. Rats weighing 100–150g and 8–12 weeks were used for the activity test. The rats were acclimatized to the experimental environment for a minimum of five days. Each rat was housed in an individual cage measuring 350cm² with a height of 18cm, lined with husks, and covered with wire mesh. The room was maintained at a temperature of 25°C with a humidity level of 60 to 70%, and a controlled lighting cycle of 12h of darkness followed by 12h of light. The diet provided consisted of standard commercial laboratory pellets at a rate of 20g per rat per day, with water available *ad libitum*. The environmental conditions were adjusted to minimize stress in the rats. Each cage was assigned a number and randomized before treatment. Standard parameters for environmental maintenance were used (Liu and Fan 2017).

Animal preparations

This study used white albino rats with healthy skin. Shaving was performed on a 6×8cm² area of the back for toxicity testing and a 4×4cm² area for activity testing, covering a total area of 10% of the experimental rats's body surface area. The rats were shaved 24h before the experiment. The anesthesia used was ketamine 40-60mg/kg BW and xylazine at a dose of 3-5mg/kg BW (Wang-Fischer 2008).

Experimental design and treatment

A completely randomized design (CRD) was used for this experimental study. OECD Guideline 402 (OECD, 2017) was used for acute dermal toxicity testing, which consisted of a pre-test step and a main test. In the pre-test, 24 rats were divided into six groups: negative control (P0), positive control (P1), 10% concentration ointment (P2), 20% (P3), 30% (P4), and 40% concentration ointment (P5). The ointment was applied once daily for 18 days, followed by UV exposure every three days.

Acute dermal toxicity

The acute dermal toxicity test consisted of two stages: the first stage was a preliminary test for determining the dosage, and the second stage was the main test. The preliminary test was carried out with one laboratory rat,

following the procedure shown in Fig. 1. Here, an initial dosage of 1000mg/kg BW was chosen as the natural ingredients were well-known. However, when the information is not available, a dose of 200mg/kg BW is taken. The ointment was rubbed into the shaved skin of the rats, which were then bandaged and covered with a plaster that did not cause irritation. The objective of this application is to provide 24h contact with the test substance and avoid consumption by rodents. After the exposure time, the plaster was removed and cleaned with distilled water to remove the residue. After exposure, observations were made at 0, 24, 48, and 72h and continued until day 14. The parameters observed include clinical assessment, weight loss, skin pathology examination, and death of the test rats. The tests are stopped if death or poisoning is detected at low doses. The main test dose selected was as high as possible to avoid mortality or severe systemic toxicity.

The primary testing procedure is shown in Fig. 2. Testing began with a predetermined dosage, which was established through preliminary assessments. Following this, two additional rats were added to each treatment and control group. In this study, the treatment group received a topical application of hibiscus simplicia ointment, while the control group received a placebo in an amount equivalent to that of the treatment group.

Observations of anatomical pathology in the treated skin areas were conducted at 24, 48, and 72h post-exposure to assess symptoms of irritation, such as erythema and oedema. An arbitrary scale of 0-4, consisting of erythema and edema scales, was used in this study. For the erythema scale, the calculation started from 0 for no erythema, 1 for very mild erythema, 2 for clearly observable erythema, 3 for moderate erythema, and 4 for severe erythema. Meanwhile, for edema, a similar scoring system was used: 0 for no edema, 1 for very mild, 2 for mild, 3 for moderate, and 4 for severe (Krismayogi et al. 2018). Furthermore, the data were determined using the primary irritation index (PII) value, calculated as the sum of all erythema and edema values at the time of observation divided by the number of rats multiplied by the number of observation times (Kuncari et al. 2015). The Draize test was used to determine the degree of irritation, which was classified as non-irritating (< 0.5), mild (0.5-2.0), moderate (2.0-5.0), and high (5.0-8.0) (Baldisserotto et al. 2018). The LD_{50} value or median lethal dose in all tests was based on the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), and its value was calculated using the Thomson-Weil test.

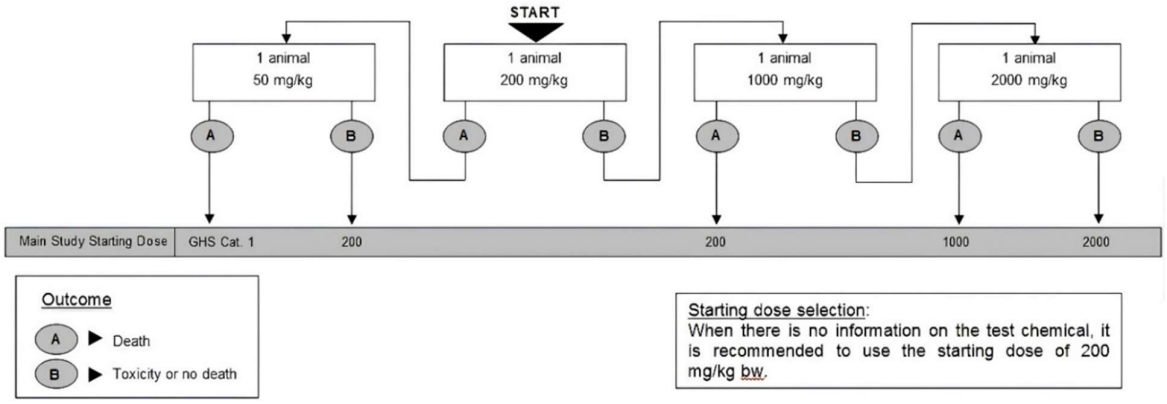

Fig. 1: Preliminary test stages (OECD 2017).

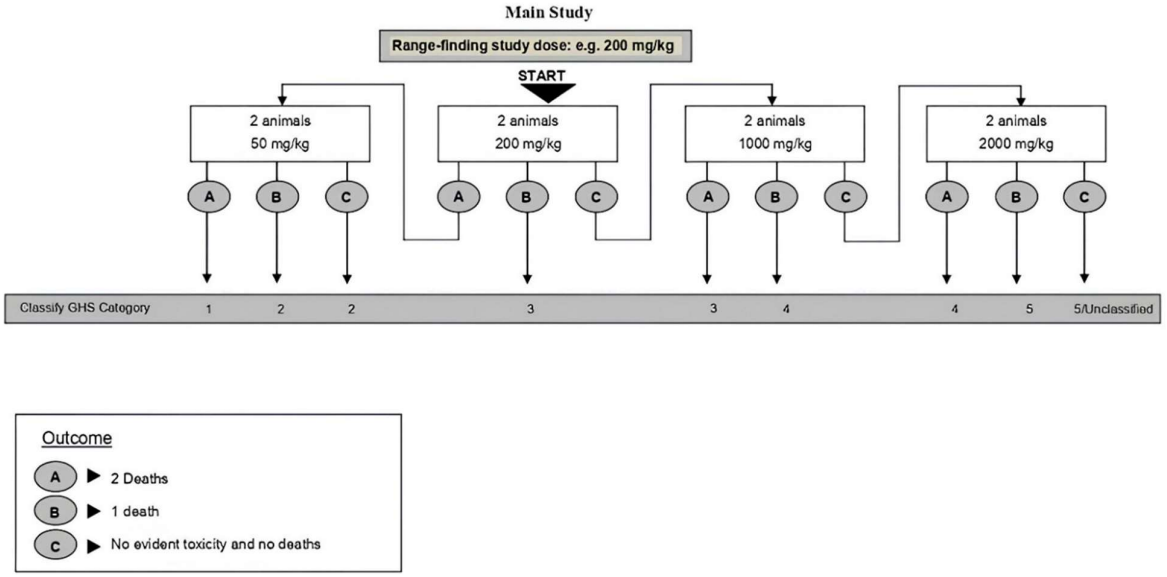

Fig. 2: Main test stages (OECD 2017).

Hair growth activity

Ten hairs were selected from the body surface of the rat using tweezers. A portion of the five longest hairs included in those collected was corrected with a Vernier calliper after being straightened upon film. Length was measured at 3-day intervals until day 18 (Purwatini et al. 2014).

Bloods sampling

Blood sampling in experimental rats was carried out in retro-ocular blood sinuses with survival (Fox 2015). The chosen sample was punctured under the eyeball on either the orbital sinus or the medial canthus towards the optic foramen under an aseptic microhaematocrit device (orbital sinus). Concurrently, the opposite end of the tube was connected to an ethylene diamine tetraacetic acid (EDTA) vacutainer tube, which served as the blood reservoir. The microhematocrit tube was rotated until the venous plexus was accessed. If the tube is rotated four times, it must be returned to its original position four times. The collected blood was immediately transferred into an EDTA vacutainer tube containing an anticoagulant solution. Subsequently, the tubes were labeled according to the treatment group and stored in a cool box.

Skin sampling

Skin samples were taken 24h after treatment. Rats were euthanized by dislocating the cervical spine so that the rats could die quickly without feeling pain. Then, a biopsy was performed on the rat's back skin, and a skin sample was taken, measuring approximately 1×1cm. After that, the skin sample was put into an organ tube containing sufficient 10% NBF (Gunawan et al. 2019).

Histological preparation

Histological preparations were performed using the Kiernan method. Tissues were fixed in 10% neutral buffered formalin (NBF) for 24h, then trimmed and processed using a tissue processor. Dehydration occurred sequentially in 70, 80, 90, and 96% alcohol, followed by two 2h stages in toluene. The tissue was subsequently blocked using liquid paraffin at a temperature of 56 degrees Celsius for two hours twice. The paraffin blocks were then sectioned into 4-5µm thick sections using a microtome. The sections of tissue were floated on water and caught with glass objects, then dried and ready to be colored with hematoxylin and eosin (HE).

Hematoxylin and eosin (HE) staining was conducted using the Harris method by immersing the glass slide preparations in xylene I, II, and III for 5mins each. Subsequently, the samples were immersed in 100% alcohol I and II for 5mins each, rinsed with distilled water, and then immersed in Harris hematoxylin for 15mins. Through the method of repeated lifting and lowering, the sample was dipped in distilled water. Next, the specimen was dipped in 1% acetic alcohol 7-10 times, soaked in distilled water for 15mins, and immersed in eosin for 2mins. The samples were then sequentially immersed in 96% alcohol I and II for 3mins, 100% alcohol I and II for 3mins, and xylene IV and V for 5mins. After that, the specimens were dried, and Entellan® was used for mounting. The samples were prepared for histological observation under a microscope.

Histological analysis

For five different microscopic fields, skin preps were examined by using a light microscope. The normal skin microscopic features were evaluated by measuring the epidermal layer thickness, hair follicle diameter, and determining damage to the hair follicle epithelium at 400X magnification. Meanwhile, counting melanocytes was done in the basal layer with the help of 1000X magnification.

Hematological analysis

The parameters monitored include red blood cells (RBC), hematocrit (HCT), white blood cells (WBC), and hemoglobin (Hb). The assessments were performed utilizing the Rayto RT-7600 automated hematology analyzer. The rats were bled from orbital veins to start the process as described previously. Whole blood in an EDTA tube was mixed and loaded into the machine. The machine's suction head sucked blood automatically.

Statistical Analysis

Nonparametric testing was used to analyze the acute toxicity test data. Data are presented as mean±SD. The Thompson-Well test with a 95% confidence level was used to analyze the LD50. Paired t-tests ($P<0.05$) were used to analyze body weight before and after treatment. Furthermore, a modified Draize test was used to analyze the irritation tests. ANOVA was used to evaluate the activity test data. The Bonferroni test was applied to analyse the hair growth observation results, followed by the Duncan test for the histological and haematological analysis results. Conclusions were drawn from the descriptive analysis of the results of each parameter.

RESULTS AND DISCUSSION

Hematology values

The hematological values of rats, including red blood cell (RBC), haematocrit (HCT), hemoglobin (Hb), and white blood cell (WBC) counts, are presented in Table 1.

Table 1: Mean ± SD hematological values of albino rats

Group	Variable treatment			
	RBC	HCT	Hb	WBC
	(10^6/µL)	(%)	(g/dL)	(10^3/µL)
P0	5.3±1.00^a	26.4±4.41^a	15.7±1.82^a	8.7±1.55^a
P1	5.0±0.44^a	24.8±1.74^a	15.0±0.73^a	17.3±4.91^b
P2	4.9±0.32^a	23.6±1.91^a	15.3±1.12^a	11.7±2.14^a
P3	4.9±0.18^a	23.4±0.63^a	15.5±0.83^a	9.6±1.69^a
P4	5.1±0.24^a	25.0±1.41^a	16.3±0.86^a	9.3±1.57^a
P5	4.7±0.76^a	23.0±3.53^a	15.0±2.52^a	9.4±1.09^a

RBC: red blood cells; HCT: Hematocrit, Hb: hemoglobin; WBC: White blood cells. P0 (negative control), P1 (positive control only exposure to UV light), P2 (hibiscus leaf simplicia ointment with 10% + UV light), P3 (hibiscus leaf simplicia ointment 20% + UV light), P4 (hibiscus leaf simplicia ointment 30% + UV light), and P5 (hibiscus leaf simplicia ointment 40% + UV light). Distinct superscript letters within the same column denote statistically significant differences among the treatment groups ($P<0.05$).

UV-exposed rats (P1) and rats treated with simplicia ointments of different concentrations (P2, P3, P4, and P5) had a lower RBC count, HCT, and Hb levels than the negative control group (P0). Nevertheless, this reduction was not statistically significant ($P>0.05$) (Table 1). This

decrease is thought to be caused by free radicals from ultraviolet light, which can damage proteins, DNA, and lipids that comprise the cell membrane (Brand et al. 2018). Erythropoietin, a glycoprotein hormone important in erythropoiesis, is also affected by free radicals due to the induction of red cell proliferation. Free radical-mediated disturbance may result in the depletion of RBC counts in the blood (Basit et al. 2020). Specifically, free radicals could be involved in erythropoiesis to stimulate erythrocytes and thus decrease the number of RBCs, which also affects the hematocrit value. A decline in erythrocytes and hematocrit, leading to a decrease in blood oxygen-carrying capacity, can result in pathological conditions such as anemia (Zuo et al. 2019). Utami et al. (2020) reported that oxidative stress induced enhanced loss of whole lipid fullness and fragility of lipid peroxidation in A-positive erythrocyte membranes, making the cells more prone to lysis. When erythrocyte membranes are ruptured, hemoglobin is released into the plasma; thus, although there is an increase in hemoglobin content, erythrocyte levels decrease because of hemolysis.

The white blood cell (WBC) counts in the positive control group (P1) were significantly higher ($P<0.05$) than those in the negative control group (P0) in rats that were only exposed to UV light and did not receive the simplicia ointment. The increase in leukocytes suggests an inflammatory reaction caused by the body's production of radicals due to UV light exposure. According to a study by Ansary et al. (2021), an inflammatory response results in leukocytosis, or an increase in the number of leukocytes that support the body's defense system. When hibiscus leaf ointment was applied, leukocyte counts did not rise. This suggests that the antioxidant activity, or the reduction of radical sources, is caused by the chemical composition of hibiscus leaves, primarily polyphenols, which in turn prevents the inflammatory response that results in leukocytosis (Pratiwi and Husni 2017; Kowti et al. 2020; Kapoor et al. 2021).

Hair growth activity test

Table 2 shows the results of hair growth and length when hibiscus leaf ointments were applied on various days.

An assay was conducted to assess the effect of different concentrations of hibiscus leaf simplicia incorporated into an ointment formulation on the acceleration of hair growth in white rats exposed to UV light. Hair-generating action was monitored in relation to average hair length. In this study, the relationship between the average hair length and dosages of ointment was briefly mentioned (Table 2): higher ointment concentrations were associated with significantly greater increases in average rat hair length. As regards the positive control, group P1 was the one that presented the smallest value of hair average length. On the other hand, rats given hibiscus leaf simplicia ointment at concentrations of 10, 20, and 30% with sunscreen had greater average hair length than the group that was treated with hibiscus leaf simplicia ointment at a concentration of 5%. Group P5 (receiving UV exposure and 40% ointment of simplicia) also revealed significantly longer average hair length in comparison with groups P1 (positive control), and P0 (negative control) on day 15th and day 18th.

The P1-related results appear to be associated with prolonged exposure to UV irradiation, which causes decreased damage to hair as reported earlier. This exposure leads to free radical formation, followed by photoaging of skin cells and additional destruction of structures within the hair. Reactive oxygen and nitrogen species (ROS/RNS) are generated following UV exposure. The major ROS produced are hydroxyl and peroxyl radicals, superoxide anions and active species such as hydrogen peroxide (H_2O_2) and singlet oxygen. At the same time, RNS generated includes nitric oxide and nitrous dioxide. Such accumulation of these free radicals, at high levels can damage the integrity and mechanism of the cells by causing degeneration (Saewan and Jimtaisong 2013).

The activity of specimens for groups P4 and P5 was not significantly different ($P>0.05$) with group P0 that potential containing in hibiscus leaf simplicia rich natural compounds can be used to enhance hair growth activity as external mucilage preparation. Flavonoids possess a chromophore group, a conjugated aromatic system capable of strongly absorbing light in the UV wavelength range, including both UVA and UVB. In addition to flavonoids, the phenolic compounds present in hibiscus leaves exhibit mutually conjugated bonds within the benzene nuclei. When subjected to UV radiation, these materials resonate, which is allowed by electron transfer reactions. Because the conjugated systems in phenolic and chemical compounds that are typical in sunscreens are similar, it is assumed that these compounds may have the ability to provide photoprotection (Prasiddha et al. 2016).

The findings of this study are consistent with those of Widyastuti et al. (2019), who confirmed that cream bath formulas with hibiscus leaf extract at concentrations of 10, 15, and 20% were the most effective for accelerating hair growth in New Zealand rabbits. In addition, Febriani et al. (2016) reported that hair tonic preparations consisting of a 2.5% and a 5% ethanol extract of hibiscus leaves might have a hair growth-stimulating effect as strong as that of

Table 2: Average hair length of white rats

Group	Average hair length (cm) ± SD					
	Day 3	Day 6	Day 9	Day 12	Day 15	Day18
P0	0.460±0.052^a	0.631±0.049^a	0.847±0.043^a	0.928±0.050^a	1.110±0.138^a	1.208±0.148^a
P1	0.161±0.007^b	0.194±0.029^b	0.250±0.027^b	0.331±0.020^b	0.420±0.053^b	0.560±0.085^b
P2	0.184±0.032^b	0.218±0.033^b	0.265±0.024^b	0.359±0.039^b	0.544±0.152^b	0.704±0.160^b
P3	0.192±0.057^b	0.232±0.070^b	0.264±0.066^b	0.434±0.083^b	0.623±0.173^b	0.817±0.135^b
P4	0.244±0.050^b	0.436±0.091^b	0.579±0.041^b	0.742±0.118^a	0.857±0.127^a	0.977±0.127^a
P5	0.302±0.095^b	0.519±0.050^a	0.736±0.086^a	0.914±0.136^a	1.127±0.151^a	1.213±0.158^a

P0 (negative control, no treatment), P1 (positive control, only UV light exposure), P2 (10% concentration ointment and UV light exposure), P3 (concentration ointment 20% and UV light exposure), P4 (30% concentration ointment and UV light exposure), P5 (40% concentration ointment and UV light exposure). Distinct superscript letters within the same column signify statistically significant differences among the treatment groups ($P<0.05$).

hair tonic formulations containing 2% minoxidil. Notably, the hair tonic based on a 10% ethanol extract of hibiscus leaves exhibited the most significant activity over the formulation containing 2% minoxidil. Among other things, flavonoids and terpenoids from hibiscus leaves are known to promote hair growth as they support the capillary wall of the blood vessels, which in turn feed the hair follicle. This support increases blood flow and provides the hair follicles with the necessary nutrients (Allayie et al. 2012). Flavonoids are polyphenolic compounds found in plants that significantly affect vascular function by enhancing blood flow and endothelial function via their vasodilatory properties (Demirel and Yilmaz 2024).

Epidermis thickness

The average thickness of the epidermis was measured to assess the effect of varying concentrations of hibiscus leaf simplicia incorporated into the ointment formulation on the epidermal layer of the skin in white rats subjected to UV light exposure. The results regarding the average epidermal thickness were derived from measurements of the epidermal layer of the skin (Fig. 3). According to the findings of this study (Table 3), a correlation was observed between the average epidermal thickness and the concentration of the ointment administered; specifically, an increase in the ointment concentration corresponded with a decrease in the average thickness of the rat skin epidermis. P1, which served as a positive control and was exposed to UV light, exhibited the highest average epidermal thickness. On the other hand, the negative control group (P0) had a lower mean epidermal thickness than the positive control group. The mean epidermal thickness was lower in the groups treated with ointments at concentrations of 10, 20, 30, and 40 %.

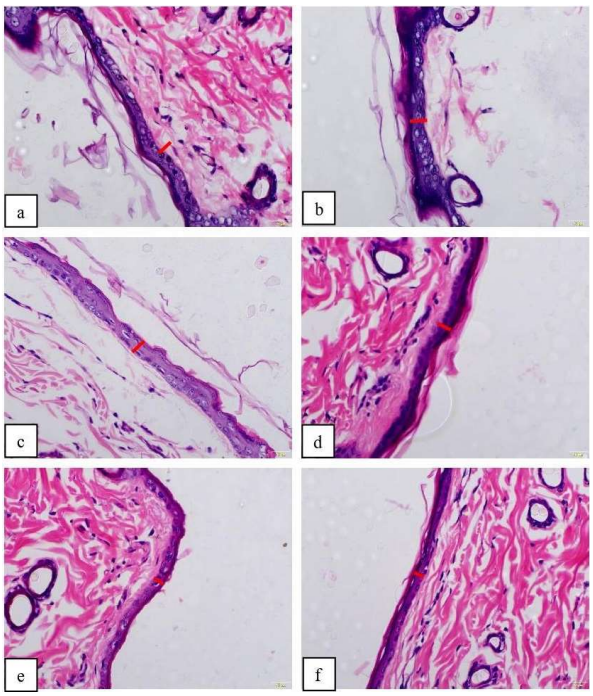

Fig. 3: Histological observation results on the epidermis of albino rats (▬) in treatments P0 (a), P1 (b), P2 (c), P3 (d), P4 (e), and P5 (f) (HE, 400X).

Table 3: Average epidermis thickness of white rats

Test group	Epidermal thickness (µm)
P0	17.875±5.810
P1	19.163±2.963
P2	17.099±2.102
P3	15.648±2.425
P4	15.102±4.608
P5	15.020±1.911

P0 (negative control, no treatment), P1 (positive control, only UV light exposure), P2 (10% concentration ointment and UV light exposure), P3 (concentration ointment 20% and UV light exposure), P4 (30% concentration ointment and UV light exposure, P5 (ointment concentration of 40% and exposure to UV light). Values (Mean±SD) did not differ in various groups.

P1 group results: The epidermis was thickened, and the skin colour darkened after UV light exposure. These findings are consistent with those of Makiyah et al. (2014), who showed that the skin epidermis mitotic index under UVC light is higher following long exposure. Likewise, Wibisono et al. (2020) found that UVB exposure thickens the epidermis in the back skin of male Wistar rats (*Rattus norvegicus*). UV light stimulates skin darkening by elevating melanocyte activity and augmenting melanin production. This damage leads to the activation of the peptide hormone ÿ-melanocyte-stimulating hormone (ÿ-MSH), which is synthesized by protein 53 (p53) in response to DNA damage (Suryani 2020). Supplements such as UV-induced production of free radicals and damage to the most superficial layer of the skin, the stratum corneum, have been reported (Makiyah et al. 2014). These free radicals stimulate the proliferation of epidermal cells, resulting in hyperplasia (Wei et al. 2024).

The ointment-treated group demonstrated epidermal thinning due to the antioxidant compounds present in the hibiscus leaf simplicia. Antioxidants are substances that inhibit the oxidation of oxidizing molecules to counteract free radicals (Wibisono et al. 2020). The chromophore group-conjugated aromatic systems are comprised of flavonoids in the hibiscus leaves. Accordingly, these compounds can absorb UV radiation. In addition to flavonoids, the phenolic compounds in hibiscus leaves possess a conjugated bond that is bonded to the benzene nucleus which can receive abstracted electrons under UV light. Phenolic compounds are known to have photoprotective properties, and standard sunscreen chemical compounds also possess a conjugation system, indicating that they may also have photoprotective activities which are very close to our findings (Prasiddha et al. 2016). Polyphenols have potent antioxidant and anti-inflammatory properties. They can protect the skin against skin damage, such as inflammation and oxidative damage. According to this study, these characteristics are essential for hindering skin aging and conserving a user's skin (Ratz-Łyko et al. 2015).

Melanocyte count

To determine the effects of hibiscus leaf simplicia on the normal amount of melanocytes in the basal layer of the epidermis of white rat skin exposed to UV light, melanocyte counts were performed. The basal layer of the epidermis contains melanocytes. The study's findings (Table 4 and Fig. 4) revealed that the number of

melanocytes was related to the amount of ointment. The ointment-treated group had fewer skin cells that produced melanin than the group exposed to UV light. The P1 group, as a positive control, and the P2 group receiving 10% ointment and UV exposure had the maximum number of melanocytes. On the contrary, the P3 and P5 groups that got the treatments with ointments at 20% and 40% had fewer melanocytes than the negative control. The lowest number of melanocytes was observed in the P4 group that received 30% ointment and UV rays.

Table 4: Average number of melanocytes in white rats

Test group	Number of melanocytes
P0	34.00±9.57
P1	39.00±6.04
P2	39.00±9.70
P3	35.25±6.94
P4	33.25±7.08
P5	34.75±7.60

The first was treatment P0 (placebo control, no treatment), P1 (positive control, only UV light exposure), P2 (ointment concentration 10% and UV light exposure), P3 (ointment concentration 20% and UV light exposure), P4 (ointment concentration 30% and UV light exposure, P5 (ointment concentration 40% and exposure to UV light). Values (Mean±SD) did not differ in various groups.

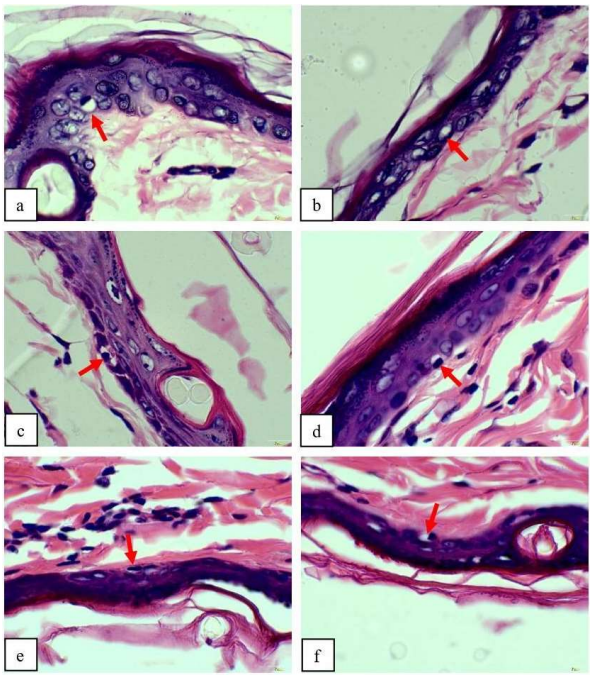

Fig. 4: Histological observation results of white rat melanocytes (red arrow) in treatments P0 (a), P1 (b), P2 (c), P3 (d), P4 (e), and P5 (f) (HE, 1000x).

The results in group P1 were due to continuous exposure to ultraviolet light, which led to an increased number of melanocytes in the epidermal layer of the skin. According to Sari et al. (2020), UV light treatment resulted in the highest number of melanocytes compared to treatment with aqueous extract of agarwood leaves (*Aquilaria macrocarpa*) and control, which also resulted in a lower number of melanocytes. UV radiation promotes melanocyte growth and movement and enhances melanin production. When the skin is exposed to UV light, it causes peroxidation of cell membrane lipids. This peroxidation creates free radicals. These radicals are known to trigger melanocyte overproduction and the release of melanin. UV light also stimulates the release of ACTH and α-MSH. This stimulates tyrosinase activity and influences melanocyte proliferation and melanin production (Sheth and Pandya 2011). More melanin means more protection from sun damage to the skin. Notably, eumelanin, a pigment that gives skin and hair their color, absorbs UV light and protects the skin. This absorption reduces the direct DNA alterations induced by UV (Huang et al. 2017; Solano 2020).

The ointment used in this group had a depigmenting effect on melanocytes. The reason behind this is due to the presence of antioxidant compounds in hibiscus leaf simplicia. Some of the active components found in hibiscus leaves are flavonoids and phenolic compounds, which act as antioxidants. Flavonoids have been found to have antioxidant activity, and plants produce them when exposed to high UVB irradiation (Jing et al. 2023). These flavonoids contain a chromophore group characterized by a conjugated aromatic system, which shows UV-light absorbing capacity. In addition to flavonoids, phenolic compounds in hibiscus leaves exhibit mutually conjugated bonds within the benzene nucleus, where exposure to UV light induces resonance through electron transfer. The similarity between the conjugation systems in phenolic compounds and those typically found in sunscreens often imparts these compounds with potential photoprotective properties (Prasiddha et al. 2016).

Hair follicle damage

Hair follicle damage was assessed by measuring the average diameter of hair follicles and observing their structure of the hair follicles. This study aimed to assess the impact of varying concentrations of hibiscus leaf simplicia incorporated into ointment formulations on the protection of hair follicles in white rats subjected to UV light exposure. The average hair follicle diameter was determined by measuring the average diameter of hair follicles. The structure of the hair follicles (HF) was obtained based on observations of the structure of hair follicles, which were described descriptively.

Data analysis showed that the diameter of hair follicles was linked to the amount of ointment administered (Table 5). In particular, an increase in the concentration of the ointments contributed to an enlargement of the average diameter of rat hair follicles. The epidermis was thinnest in the positive control (P1) group, which was irradiated with UV light. The mean hair follicle diameter in the P2 and P3 groups (10% and 20% ointment, respectively) exposed to UV light did not exceed that of the positive control but was lower than that of the negative control (P0). Furthermore, the size of the HF in Groups P4 (30% ointment) and P5 (40% ointment), both under UV light, was larger than that of the negative control. These results are consistent with those reported by Putra et al. (2020), who reported that a leaf ethanol extract of hibiscus changes the murine skin for up to 25 days and produces remarkable hair growth. Likewise, the increase in hair growth was greater for the extract concentrations of 10% and below.

Table 5: Average diameter of white rat hair follicles

Test group	Hair follicle diameter (μm)
P0	23.652±2.314
P1	17.555±2.501
P2	17.721±2.441
P3	18.270±2.852
P4	19.361±1.274
P5	19.683±2.999

Description: P0 (negative control, no treatment), P1 (positive control, only UV light exposure), P2 (10% concentration ointment and UV light exposure), P3 (concentration ointment 20% and UV light exposure), P4 (30% concentration ointment and UV light exposure, P5 (ointment concentration of 40% and exposure to UV light). Values (Mean±SD) did not differ in various groups.

The data on hair follicle structure (Table 6 and Fig. 5) showed that the structure of hair follicles depended on the intensity of the ointment used. Compared to the group exposed only to UV light, the ointment users had healthier hair follicles. The follicles were oval-round, and the P0 group had a complex squamous epithelium; cell layers were formed by two to four cells. The morphology of P1 was oval, accompanied by a complex squamous layer and 1-2 cells. An oval-shaped follicle and papillary folds with complex squamous epithelial cells (1-3 cells) were characteristically observed in the P2 group. The P3 group had an oval shape. They are composed of two to three squamous epithelial cells. The challenged follicles in the P4 group were either spherical or elliptical. The composition is 2–3 complicated squamous epithelial cells. Finally, the P5 group showed oval-shaped follicles with complex linings of 2-3 squamous epithelial cells.

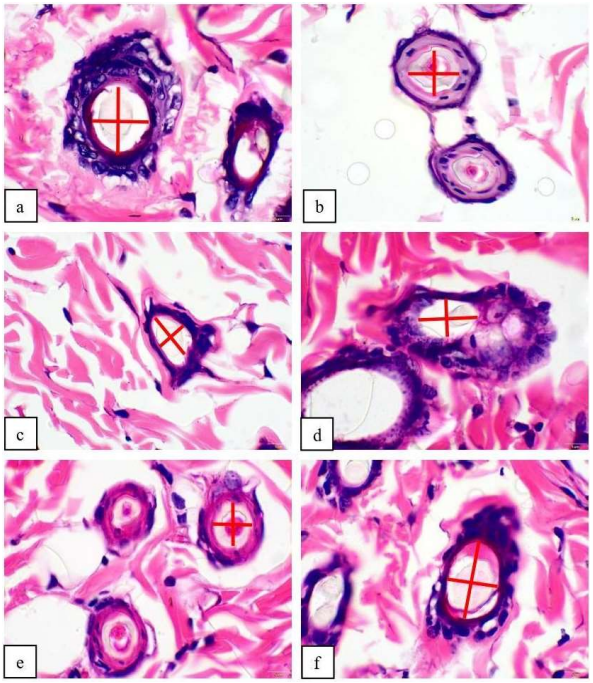

Fig. 5: Results of histological observations of white rat hair follicles (+) in treatments P0 (a), P1 (b), P2 (c), P3 (d), P4 (e), and P5 (f) (HE, 1000x).

Chronic long-term exposure to UV light was found in group P1 in this study, as evidenced by the shrinkage and structural damage of hair follicles. This damage is caused by the production of free radicals, in which free radicals from UV radiation can trigger skin aging (Pratama et al. 2020). With aging, hair transplantation is believed to be related to the contraction or shrinking of hair follicles in the skin (Fernandez-Flores et al. 2019). UVA radiation can reach and harm the cells in the dermis layer of the skin, which can cause changes to the complexion when exposed for too long. There can also be degradation of collagen, which may result in premature aging of the skin and photoaging (Lan 2019).

The mean diameter of the follicles in the hibiscus ointment-treated group was increased, and the hair follicular structures more regular when compared with those from the control group. This may be attributed to the antioxidant action of the hibiscus simplicia leaves. For instance, flavonoids of hibiscus leaf can be effective on absorbing strong light in the UVA and UVB regions (Prasiddha et al. 2016). Moreover, flavonoids and terpenoids can toughen the capillaries that feed the hair roots, meaning more blood flow to carry nutrients to hair follicles and maintain healthy hair in line with the nutritional needs of hair (Allayie et al. 2012). In addition, Al-Snafi (2018) noted that the leaf of hibiscus preparation exhibited very good hair growth activity increased size of hair follicle.

Acute dermal toxicity test

During the initial phase of this study, a dose of 1000mg/kg BW was administered to one rat per dose. This dosage was chosen because of the extensive literature on the chemical composition of hibiscus leaves. The results demonstrated that the concentration was safe, and there was no evidence of pain, body weight reduction, or mortality, as per the OECD guidelines 402 (OECD 2017). Thus, we performed the present study using a dose of 2000mg/kg BW, which suggested that the use of the sample was safe. Therefore, a dose of 2000mg/kg BW was selected for the first attempt at assessing acute dermal toxicity. These results are consistent with those of Mondal et al. (2016), who reported on the ethanol extracts of *H. rosa-sinensis* L. leaves. They proved to be a remarkably safe agent as shown by their acute oral toxicity studies (2000mg/kg BW) in which no adverse effects were observed.

In the primary test, experimental rats showed reduced activity, restlessness, lethargy, and decreased locomotion in search of available cues at exposure onset. However, their locomotor activity came back to pre-stress levels after 24 h post-exposure (0th hour of observation period) and stayed constant until day 14. None of the animals showed signs of toxicity; no behavioral changes, tremors, seizures, or diarrhea were recorded. The mucous membranes, the eyes, and the respiratory system were normal. There were no deaths among the treatment or control groups (mortality 0%). Specifically, erythema was observed at hour 0 to day 3 of observation, and it disappeared by day 4 of observation. The Draize test was used to assess the degree of irritation. Hair regrowth commenced on day 2 in all experimental rats and was nearly complete by day 14, although some areas with shorter hair were noted.

The results of this study are consistent with those of Sithambaram and Othman (2015) regarding the dermal acute toxicity assessment of noni fruit extract. It was found that administering doses of 2000mg/kg BW and 5000 mg/kg BW did not lead to any noticeable toxicity

Table 6: Histological structure of white rat hair follicles

Group	Histological structure of hair follicles
P0	The follicle shape is oval-round, and the cell shape is squamous epithelium complex, consisting of 2-4 cells
P1	Oval follicle shape, squamous epithelial cell shape complex, consisting of 1-2 cells
P2	Oval follicle shape, squamous epithelial cell shape complex, consisting of 1-3 cells
P3	Oval follicle shape, squamous epithelial cell shape complex, consisting of 2-3 cells
P4	The follicle shape is oval-round, and the cell shape is squamous epithelium complex, consisting of 2-3 cells
P5	Oval follicle shape, squamous epithelial cell shape complex, consisting of 2-3 cells

Description: P0 (negative control, no treatment), P1 (positive control, only UV light exposure), P2 (10% concentration ointment and UV light exposure), P3 (20% concentration ointment and UV light exposure), P4 (ointment 30% concentration and UV light exposure, P5 (40% concentration ointment and UV light exposure).

symptoms, and the mortality rate remained at zero. Merdana et al. (2020) also stated that there were no deaths in their study on the possible acute dermal toxicity of Rajas oil. When the experiment was conducted on the 3rd day, hair regrowth in the test animal reversed, and by the 14th day, it was found to be similar to the pre-treated stage hair, with slightly reduced length compared to the original.

The skin was examined for anatomical pathology at 0, 24, 48, and 72h and for the presence of erythema. Each observation period was scored according to the observations. The test results of the Primary Index of Irritation (PII) showed that the values in the control and treatment groups were not significantly different and belonged to mild irritation. We believe that the inflammatory condition seen in the control group (which had red patches on their skin) also helped rule out false positives. This should not have happened if only Vaseline had been applied.

The erythema depicted in this study might have occurred because of a deviation from the normal skin microflora and, hence, increased sensitivity of the skin. There is a relationship between normal cutaneous microbiota, skin barrier functions, and the sensitivities of the human skin, which also affects the influence of cosmetics on these three parameters (Seite and Misery 2018). In addition, hair removal performed in the laboratory can cause abrasions that induce erythema. Such a process could result in injury, which would disrupt the barrier and make it more permeable. This condition increases the transdermal absorption of the ointment, as is also anticipated from any topical ointment preparations, according to Hakim et al. (2018).

The results of weight measurements taken before treatment until day 14 showed no significant weight loss. This indicates that systemic toxicity did not occur (Merdana et al. 2020). The results of the paired t-test analysis revealed no statistically significant difference ($P>0.05$) in body weight between the control and treatment groups. According to Sithambaram and Othman (2015), who studied noni fruit extract, and Banerjee et al. (2013), who studied transdermal patches for the prevention of anatoxin-A poisoning. Generally, alterations in body weight in rats can serve as an indicator of mortality due to exposure to toxic substances. Such changes also signify the presence of side effects from drugs or chemicals, particularly if there is a reduction of more than 10% from the initial weight (Sithambaram and Othman 2015).

Around the sixth week of observation, virtually all experimental rats lost weight. This is probably due to the stress of bandaging and application, as well as removal of the dressings 24 h after exposure to the test substance. Qu et al. (2020) reported that the stress-reducing relationship between restraint and weight loss persists in the face of repeated acute stress's chronic effects on body weight (i.e., not resulting from hypophagia alone, which is also a consequence of stress). The findings of Sari et al. (2016) supported this further, reporting that the acute dermal toxicity from areca nut extract administered at 15,000mg BW was down on day two of observation. As a result of the treatment, the decrease was ascribed to the stress caused by treatment wrapping.

In this study, no signs of toxicity or fatalities were observed, and the fur of the rats grew back after the observation period. The Primary Irritation Index (PII) was recorded at 0.75 for the control group and 1.00 for the treatment group, which was classified as slightly irritating according to the Amended Draize Test. However, the PII findings were deemed invalid because of the false positives. Consequently, we verified that the hibiscus leaf (*Hibiscus rosa-sinensis* L.) ointment was non-irritating, as the PII value did not significantly differ from that of the control group. The acute dermal LD_{50} value could not be established using the Thompson-Weil method because no deaths occurred during the experiment; thus, only a pseudo-LD_{50} (Irawan et al. 2005) was determined, representing the maximum dose that can still be administered to test rats (Muhtadi 2011). According to the globally harmonized system (GHS), a 40% hibiscus leaf ointment falls under category 5 (not classified) with an acute dermal LD_{50} value exceeding 2000mg/kg BW, suggesting a potential risk if it contacts the skin (OECD 2017).

Conclusion

Simplicia ointment of hibiscus leaves (*Hibiscus rosa-sinensis* L.) in white rats (*Rattus norvegicus*) after exposure to ultraviolet light can maintain the number of erythrocytes, hematocrit value, hemoglobin level, and leukocyte count, and increase hair growth activity. The application of hibiscus leaf ointment suppressed the increase in leukocyte count, thereby reducing inflammation. Hibiscus leaf ointment at concentrations ranging from 10% to 30% significantly enhanced hair growth. Notably, a concentration of 40% resulted in longer hair compared to the negative control on days 15 and 18. However, the administration of simplicia ointment from hibiscus leaves (*Hibiscus rosa-sinensis* L.) did not maintain the normal histology of rat skin exposed to ultraviolet light. Based on the acute dermal toxicity test, the acute dermal LD50 was >2000mg/kg BW. The pseudo-LD_{50} dermal value exceeding 2000mg/kg BW categorizes this herb as unclassified, indicating that hibiscus ointment is relatively safe for dermal use.

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Conflict of Interest: The authors declare no conflicts of interest in the publication of this paper.

Data Availability: All data generated in this study are comprehensively presented in this article.

Ethics Statement: The Faculty of Veterinary Medicine Animal Ethics Committee at Udayana University (UNUD) approved this study involving experimental animals, as shown by Certificate Number: B/1/UN14.2.9/PT.01.04/2023. The Laboratory of Physiology, Pharmacology, and Veterinary Pharmacy, FKH, UNUD, was where the research was done. Hematological and histopathological preparations were performed at the Denpasar Veterinary Special Service (BBVet) in Bali, Indonesia. Histological analysis was conducted at the Laboratory of Histology, Parasitology, and Veterinary Pathology, FKH, UNUD. Histological examinations were conducted at the Laboratory of Histology, Parasitology, and Veterinary Pathology, FKH, UNUD.

Author's Contribution: I Wayan Sudira conceptualized the study, supervised the research, and reviewed the manuscript. I Gusti Ngurah Sudisma contributed to the study conceptualization, supervision, and feedback. Made Gede Adi Surya Saputra, I Gusti Bagus Aryanta Kusuma Putra, and Wayan Gede Ananta Brahmananda performed all experimental procedures and carried out data analysis and interpretation. Samsuri, Ni Luh Eka Setiasih, Anak Agung Sagung Kendran, I Made Merdana, and Ida Bagus Oka Winaya reviewed and revised the final version of the manuscript and provided feedback. I Putu Cahyadi Putra drafted and formatted the manuscript accordingly.

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REFERENCES

Agrawal R, Jurel P, Deshmukh R, Harwansh RK, Garg A, Kumar A, Singh S, Guru A, Kumar A and Kumarasamy V, 2024. Emerging trends in the treatment of skin disorders by herbal drugs: traditional and nanotechnological approach. Pharmaceutics 16(7): 869. https://doi.org/10.3390/pharmaceutics16070869

Ajiboye BO, Famusiwa CD, Nifemi DM, Ayodele BM, Akinlolu OS, Fatoki TH and Oyinloye BE, 2024. Nephroprotective effect of Hibiscus sabdariffa leaf flavonoid extracts via KIM-1 and TGF-1β signaling pathways in streptozotocin-induced rats. ACS Omega 9(17): 19334–19344. https://doi.org/10.1021/acsomega.4c00254

Aldrian E, Kusumaningtyas SDA, Supari, Nuryanto DE and Sopaheluwakan A, 2022. Technical and infrastructure modality for extreme climate early warning in Indonesia. In: Extreme natural events: Sustainable solutions for developing countries. Springer Nature, Singapore, pp: 23–49. https://doi.org/10.1007/978-981-19-2511-5_2

Allayie SA, Hemalatha S, Elanchezhiyan C, Manoharan V, Balasubramanian K and Sheikh BA, 2012. In vivo evaluation of hair growth potential of fresh leaf extracts of *Naringi crenulata*. Journal of Clinical and Experimental Dermatology Research 3(2): 1-4. https://doi.org/10.4172/2155-9554.1000148

Al-Snafi AE, 2018. Chemical constituents, pharmacological effects and therapeutic importance of *Hibiscus rosa-sinensis* – A review. IOSR Journal of Pharmacy 8(7): 101–119. http://med.utq.edu.iq/wp-content/uploads/sites/7/2021/07/chemical-constituents-pharmacological-effects-and-therapeutic-importance-of-*Hibiscus-rosa-sinensis*-a-review.pdf

Amali FW, Prajoko S and Ramadani SD, 2023. Ethnomedicine study of medicinal plants in the Menoreh Salaman village community as a biology reference book. Bioedukasi 21(2): 166. https://doi.org/10.19184/bioedu.v21i2.38863

Amtaghri S, Qabouche A, Slaoui M and Eddouks M, 2023. A comprehensive overview of Hibiscus rosa-sinensis L.: its ethnobotanical uses, phytochemistry, therapeutic uses, pharmacological activities, and toxicology. Endocrine, Metabolic & Immune Disorders Drug Targets 24(1): 86. https://doi.org/10.2174/1871530323666230522113405

Amtaghri S, Qabouche A, Slaoui M and Eddouks M, 2024. A comprehensive overview of *Hibiscus rosa-sinensis* L.: Its ethnobotanical uses, phytochemistry, therapeutic uses, pharmacological activities, and toxicology. Endocrine, Metabolic & Immune Disorders, Drug Targets 24(1): 86–115. https://doi.org/10.2174/1871530323666230522113405

Ansary TM, Hossain MR, Kamiya K, Komine M and Ohtsuki M, 2021. Inflammatory molecules associated with ultraviolet radiation-mediated skin aging. International Journal of Molecular Science 22(8): 3974. https://doi.org/10.3390/ijms22083974

Ayad R, Lefahal M, Makhloufi EH and Akkal S, 2023. Photoprotection strategies with antioxidant extracts: a new vision. Physical Sciences Reviews 9(6): 2273. https://doi.org/10.1515/psr-2022-0313

Baldisserotto A, Buso P, Radice M, Dissette V, Lampronti I, Gambari R, Manfredini S and Vertuani S, 2018. *Moringa oleifera* leaf extracts as multifunctional ingredients for "natural and organic" sunscreens and photoprotective preparations. Molecules 23(3): 664. https://doi.org/10.3390/molecules23030664

Banerjee S, Chattopadhyay P, Ghosh A, Pathak MP, Singh S and Veer V, 2013. Acute dermal irritation, sensitization, and acute toxicity studies of a transdermal patch for prophylaxis against (±) anatoxin-A poisoning. International Journal of Toxicology 32(4): 308–313. https://doi.org/10.1177/10915818134899

Barvaliya M, Chetan A, Chandan N, Ray SK, Hegde HV, Unger BS, Patel T and Roy S, 2023. Suspected cutaneous adverse drug reactions reported with traditional medicines: analysis

of data for United Nations Asia region from WHO VigiBase. Frontiers in Pharmacology 14: 1088841. https://doi.org/10.3389/fphar.2023.1088841

Basit I, Aziz A, Azam R, Aziz H, Shabbir S, Fatima N, Liaqat K and Fatima R, 2020. Influence of oxidative stress on erythrocyte's formation and function resulting in anemia. Journal of Nursing and Health Science 9(4): 37–43. https://doi.org/10.9790/1959-0904013743

Brand RM, Wipf P, Durham A, Epperly MW, Greenberger JS and Falo LD Jr, 2018. Targeting mitochondrial oxidative stress to mitigate UV-induced skin damage. Frontiers in Pharmacology 9: 920. https://doi.org/10.3389/fphar.2018.00920

Chakraborty A, Bhattacharjee A, Mondal B, Chakraborty M and Mukhopadhyay G, 2023. 5α-reductase inhibitory potential of Hibiscus rosa-sinensis: effective for the management of alopecia. Journal of Natural Remedies 23(2): 467–474. https://doi.org/10.18311/jnr/2023/31180

Demirel S and Yilmaz DA, 2024. Effects of flavonoids on vascular activity. Global Translational Medicine 3(2): 2458. https://doi.org/10.36922/gtm.2458

Farris PK and Valacchi G, 2022. Ultraviolet light protection: is it really enough? Antioxidants 11(8): 1484. https://doi.org/10.3390/antiox11081484

Febriani A, Elya B and Jufri M, 2016. Uji aktivitas dan keamanan hair tonic ekstrak daun kembang sepatu (Hibiscus rosa-sinensis) pada pertumbuhan rambut kelinci (Indonesian). J Farmacy Indonesia 8(1): 259-270.

Fernandez-Flores A, Saeb-Lima M and Cassarino DS, 2019. Histopathology of aging of the hair follicle. Journal of Cutaneous Pathology 46(7): 508–519. https://doi.org/10.1111/cup.13467

Fox JG, 2015. Laboratory animal medicine: Historical prospective. In: Laboratory Animal Medicine. Elsevier, pp: 1-21

Gambacurta A, Tullio V, Savini I, Catani MV and Gasperi V, 2024. In vitro cell-based MTT and crystal violet assays for drug toxicity screening. Methods in Molecular Biology 293. https://doi.org/10.1007/978-1-0716-4003-6_14

Giannone C, Bovo M, Ceccarelli M, Torreggiani D and Tassinari P, 2023. Review of the heat stress-induced responses in dairy cattle. Animals 13(22): 3451. https://doi.org/10.3390/ani13223451

Gunawan SA, Berata IK and Wirata IW, 2019. Histopathology of skin in recovery incision wound in rat post giving extracellular matrix (ECM) from pork's vesica urinaria. Indonesian Medical Veterinary Journal 8(3): 313–324. https://doi.org/10.19087/imv.2019.8.3.313

Hakim ZR, Purbarini KA and Tjiptasurasa TT, 2018. Uji iritasi akut dermal pada hewan uji kelinci albino terhadap sediaan body lotion ekstrak kulit biji pinang (Areca catechu L.). Farmaka 18(1): 1–13. https://doi.org/10.24198/jf.v18i1.18513.g12574

Huang Y, Li Y, Hu Z, Yue X, Proetto MT, Jones Y and Gianneschi NC, 2017. Mimicking melanosomes: polydopamine nanoparticles as artificial microparasols. ACS Central Science 3(6): 564–569. https://doi.org/10.1021/acscentsci.6b00230

Irawan ED, KS Lor and Untari S, 2005. Uji toksisitas akut dari ekstrak etanol herba sereh (Andropogon citratus DC) pada mencit putih sebagai bahan pembelajaran toksikologi. Jurnal Pengembangan Pendidikan 2(2): 210–221. https://junej.jurnal.unej.ac.id/index.php/JP2/article/download/836/650

Iriani NA, Dwiranti A and Salamah A, 2020. Indeks mitosis pucuk daun Hibiscus rosa-sinensis L. variasi Single Pink pada beberapa variasi waktu. Al-Kauniyah Journal of Biology 13(1): 1–8. https://doi.org/10.15408/kauniyah.v13i1.9454

Jing Y, Watanabe M, Aarabi F, Fernie AR, Borghi M and Tohge T, 2023. Cross-species metabolomic analyses in the Brassicaceae reveals common responses to ultraviolet-B exposure. Plant Cell Physiology 64(12): 1523–1533. https://doi.org/10.1093/pcp/pcad085

Jităreanu A, Trifan A, Vieriu M, Caba IC, Mârțu I and Agoroaei L, 2022. Current trends in toxicity assessment of herbal medicines: a narrative review. Processes 11(1): 83. https://doi.org/10.3390/pr11010083

Kabous KE, Kabous SE, Mechkirrou L, Slamini M and Iskandar SS, 2025. Antioxidants in dermatology: protecting and enhancing skin health. E3S Web of Conferences 632: 1017. https://doi.org/10.1051/e3sconf/202563201017

Kapoor M, Kaur G, Kaur N, Sharma C, Batra K and Singh D, 2021. The traditional uses, phytochemistry and pharmacology of genus Hibiscus: a review. European Journal of Medicinal Plants 32(4): 1–37. https://doi.org/10.9734/EJMP/2021/v32i430382

Kowti R, Majumder P, Harshitha HS, Joshi V, Kumar R and Ahmed SS, 2020. Hibiscus herbs – a comprehensive botanical, chemical and biological overview. World Journal of Advanced Research and Reviews 7(3): 291–303. https://doi.org/10.30574/wjarr

Krismayogi GA, Ratnayanti ID, Linawati NM, Wiryawan IS, Sugiritama IW and Wahyuniari IAI, 2018. Purple cabbage extract cream effect on erythema score of male Wistar rats' back skin exposed to UV-B radiation. Biomedical and Pharmacological Journal 11(1): 343–351. https://doi.org/10.13005/bpj/1378

Kuncari ES, Iskandarsyah I and Praptiwi P, 2015. Uji iritasi dan aktivitas pertumbuhan rambut tikus putih: efek sediaan gel apigenin dan perasan herba seledri (*Apium graveolens* L.). Media Penelitian dan Pengembangan Kesehatan 25(1): 15–22. https://doi.org/10.22435/mpk.v25i1.4092.15-22

Lan CE, 2019. Effects and interactions of increased environmental temperature and UV radiation on photoageing and photocarcinogenesis of the skin. Experimental Dermatology 28: 23–27. https://doi.org/10.1111/exd.13818

Lee S, Lee NY, Choi SH, Oh C, Won GW, Bhatta MP, Moon JH, Lee C, Kim JH, Park J and Park J, 2023. Molecular mechanism of the anti-inflammatory and skin protective effects of Syzygium formosum in human skin keratinocytes. Food Science and Biotechnology 33(3): 689. https://doi.org/10.1007/s10068-023-01380-4

Liu E and Fan J, 2017. Fundamentals of Laboratory Animal Science. CRC Press.

Makiyah SNN, Iszamriach R and Nofariyandi A, 2014. Ultraviolet c exposure increases the white pulp diameter of spleen and epidermal mitotic index of mice skin. Jurnal Kedokteran Brawijaya 28(1): 17–21. https://doi.org/10.21776/ub.jkb.2014.028.01.4

Merdana IM, Azizati STKN, Arjana AAG, Adi A and Sudira IW, 2020. Assessment of the acute dermal toxicity potential of natural veterinary medicine minyak rajas in wistar rats. Buletin Veteriner Udayana 12(2): 205–214. https://doi.org/10.24843/bulvet.2020.v12.i02.p16

Missoum A, 2018. An update review on *Hibiscus rosa-sinensis* phytochemistry and medicinal uses. Journal of Ayurvedic and Herbal Medicine 4(3): 135–146.

Mondal S, Ghosh D, Sagar N and Ganapaty S, 2016. Evaluation of antioxidant, toxicological and wound healing properties of Hibiscus rosa-sinensis L. (Malvaceae) ethanolic leaves extract on different experimental animal models. Indian Journal of Pharmaceutical Education and Research 50(1): 620–637. https://doi.org/10.5530/ijper.50.4.15

Mugale MN, Dev K, More BS, Mishra VS, Washimkar KR, Singh KP, Maurya R, Rath SK, Chattopadhyay D and Chattopadhyay N, 2024. A comprehensive review on preclinical safety and toxicity of medicinal plants. Clinical Complementary Medicine and Pharmacology 4(1): 100129. https://doi.org/10.1016/j.ccmp.2024.100129

Muhtadi M, 2011. Acute toxicity testing of extracts combination of meniran herbs (Phyllanthus niruri auct. Non L.), tempuyung leaves (*Sonchus arvensis* L.) and black cumin seeds (*Nigella sativa* L.). Jurnal Farmasi Indonesia 12(2): 69–72. https://doi.org/10.23917/pharmacon.v12i2.35

Negi S, Kumar S and Singh A, 2023. Preclinical in vivo drug development studies: limitations, model organisms, and techniques. Springer Nature, Singapore, pp: 149. https://doi.org/10.1007/978-981-19-7952-1_6

OECD, 2017. Test No. 402: OECD guideline for the testing of chemicals – Acute dermal toxicity: Fixed dose procedure. OECD Publishing, Paris.

Paudyal V, Sun S, Hussain R, Abutaleb MH and Hedima EW, 2022. Complementary and alternative medicines use in COVID-19: a global perspective on practice, policy and research. Research in Social and Administrative Pharmacy 18(3): 2524–2528. https://doi.org/10.1016/j.sapharm.2021.05.004

Prasiddha IJ, Laeliocattleya RA, Estiasih T and Maligan JM, 2016. The potency of bioactive compounds from corn silk (Zea mays L.) for the use as a natural sunscreen: A review. Jurnal Pangan dan Agroindustri 4(1): 40–45.

Pratama GMCT, Hartawan IGN, Indriani IGAT, Yusrika MU, Suryantari SAA and Sudarsa PSS, 2020. Potency of *Spirulina platensis* extract as sunscreen on ultraviolet B exposure. Journal of Medical Health 2(6):205-217. https://doi.org/10.28932/jmh.v2i6.2484

Pratiwi S and Husni P, 2017. Artikel tinjauan: potensi penggunaan fitokonstituen tanaman Indonesia sebagai bahan aktif tabir surya. Farmaka 15(4): 18–25. https://doi.org/10.24198/jf.v15i4.14581.g7430

Purwatini I, Munawaroh R and Darwati N, 2014. Kombinasi daun teh dan mangkokan sebagai penumbuh rambut. Universitas Gadjah Mada, Yogyakarta.

Putra IB, Jusuf NK and Sumantri IB, 2020. The potency of Hibiscus rosa-sinensis Linn. leaves ethanol extract as hair growth. Macedonian Journal of Medical Sciences 8(A): 89–92. https://doi.org/10.3889/oamjms.2020.4211

Qu N, He Y, Wang C, Xu P, Yang Y, Cai X, Liu H, Yu K, Pei Z and Hyseni I, 2020. A POMC-originated circuit regulates stress-induced hypophagia, depression, and anhedonia. Molecular Psychiatry 25(5): 1006–1021. https://doi.org/10.1038/s41380-019-0506-1

Rajesham VV, Raghavendra M, Supriya Reddy G, Roshan Ali P and Rama Rao T, 2024. Evaluation of Hibiscus sabdariffa aerial parts against pyloric ligation-induced ulcers in experimental rats. Journal of Natural Remedies 24(5): 1135–1140. https://doi.org/10.18311/jnr/2024/35163

Rambe PS, Putra IB and Yosi A, 2022. The effect of roselle leaf (*Hibiscus sabdariffa* L.) extract gel on wound healing. Journal of Medical Life 15(10): 1246. https://doi.org/10.25122/jml-2021-0425

Ratz-Łyko A, Arct J, Majewski S and Pytkowska K, 2015. Influence of polyphenols on the physiological processes in the skin. Phytotherapy Research 29(4): 509–517. https://doi.org/10.1002/ptr.5289

Saewan N and Jimtaisong A, 2013. Photoprotection of natural flavonoids. Journal of Applied Pharmaceutical Science 3(9): 129–141. https://doi.org/10.7324/JAPS.2013.3923

Sari DI, Rahmawanty D, Jultan Y and Naba SS, 2020. Sediaan ekstrak air daun gaharu (Aquilaria microcarpa) memiliki potensi memperbaiki kulit yang terpapar sinar ultraviolet. Journal of Pharmascience 7(1): 36–42. https://doi.org/10.20527/jps.v7i1.8071

Sari LM, Suyatna FD, Subita GP and Auerkar EI, 2016. Acute dermal toxicity study of Areca catechu Linn. extract in Sprague-Dawley rats. Asian Journal of Pharmacology and Clinical Research 9(9): 209. https://doi.org/10.22159/ajpcr.2016.v9s3.14462

Scotti L and Scotti MT, 2025. ADME properties in drug delivery. Pharmaceutics 17(5): 617. https://doi.org/10.3390/pharmaceutics17050617

Seite S and Misery L, 2018. Skin sensitivity and skin microbiota: is there a link? Experimental Dermatology 27(9): 1061–1064. https://doi.org/10.1111/exd.13686

Sheth VM and Pandya AG, 2011. Melasma: a comprehensive update: part II. Journal of the American Academy of Dermatology 65(4): 699–714. https://doi.org/10.1016/j.jaad.2011.06.001

Sithambaram S and Othman HH, 2015. Acute and sub-acute dermal toxicity studies of Morinda citrifolia L. fruit extract in Sprague-Dawley rats. Asian Journal of Pharmaceutical and Clinical Research 8: 400–408.

Solano F, 2020. Photoprotection and skin pigmentation: melanin-related molecules and some other new agents obtained from natural sources. Molecules 25(7): 1537. https://doi.org/10.3390/molecules25071537

Suryani A, 2020. Faktor-faktor yang memengaruhi pigmentasi manusia. Cermin Dunia Kedokteran 47(9): 682–685. https://doi.org/10.55175/cdk.v47i11.1195

Tang X, Yang T, Yu D, Xiong H and Zhang S, 2024. Current insights and future perspectives of ultraviolet radiation (UV) exposure: friends and foes to the skin and beyond the skin. Environment International 185: 108535. https://doi.org/10.1016/j.envint.2024.108535

Utami LPN, Rusmini H, Nurmalasari Y and Hermawan D, 2020. Pengaruh vitamin D3 terhadap glukosa darah tikus Wistar yang dipapar asap rokok. Arteri: Jurnal Ilmu Kesehatan 1(2): 131–139. https://doi.org/10.37148/arteri.v1i2.52

Verma A, Zanoletti A, Kareem KY, Adelodun B, Kumar P, Ajibade FO, Silva LFO, Phillips AJ, Thangathurai K, Bontempi E and Dwivedi A, 2023. Skin protection from solar ultraviolet radiation using natural compounds: a review. Environmental Chemistry Letters 22(1): 273–295. https://doi.org/10.1007/s10311-023-01649-4

Wang X, Ma Y, Xu Q, Shikov AN, Pozharitskaya ON, Flisyuk EV, Liu M, Li H, Vargas-Murga L and Duez P, 2022. Flavonoids and saponins: what have we got or missed? Phytomedicine 109: 154580. https://doi.org/10.1016/j.phymed.2022.154580

Wang-Fischer Y, 2008. Anatomy and cerebral circulation of the rat. Manual of Stroke Models in Rats. CRC Press, pp: 13-24

Wei M, He X, Liu N and Deng H, 2024. Role of reactive oxygen species in ultraviolet-induced photodamage of the skin. Cell Division 19(1): 1. https://doi.org/10.1186/s13008-024-00107-z

Wibisono EW, Ratnayanti IGAD, Arijana IGKN and Linawati NM, 2020. Krim ekstrak etanol bawang putih tunggal (Allium sativum) menghambat penebalan epidermis tikus Wistar jantan (Rattus norvegicus) yang dipapar sinar ultraviolet-B. Jurnal Kesehatan Andalas 9(1). https://doi.org/10.25077/jka.v9i1.1275

Widyastuti L, Ningsih D and Aisiyah S, 2019. Pengaruh pemberian sediaan creambath ekstrak daun kembang sepatu (*Hibiscus rosa-sinensis*) pada pertumbuhan rambut kelinci (New Zealand). Journal of Farmasi 8(1): 15–21. https://doi.org/10.37013/jf.v1i8.75

Zuo L, Prather ER, Stetskiv M, Garrison DE, Meade JR, Peace TI and Zhou T, 2019. Inflammaging and oxidative stress in human diseases: from molecular mechanisms to novel treatments. International Journal of Molecular Science 20(18): 4472. https://doi.org/10.3390/ijms20184472