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Original Article

Anti-Aging Efficacy of Mangrove Leaf Extract Cream Assessed by SPF and Collagen Density Enhancement: *In Vitro* and *In Vivo* Approaches

I Gusti Agung Ayu Kusuma Wardani[1*], Ketut Agus Adrianta[1], Ni Nyoman Wahyu Udayani[1], Meriana Barreto Amaral[2], Ni Made Dharma Shantini Suena[3]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar-Bali, 80233, Indonesia
[2]Departamento Escola Superior de Parteira, Faculdade De Medicina E Ciências De Saúde, Universidade Nacional Timor Lorosae, Rua Avenida de Caicoli Dili
[3]Department of Pharmaceutics, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar-Bali, 80233, Indonesia

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Correspondence:
I Gusti Agung Ayu Kusuma Wardani
kusumawardani@unmas.ac.id

Abstract

Background: Excessive ultraviolet (UV) exposure is a major contributor to skin photoaging through oxidative stress, inflammation, and collagen degradation. Mangrove leaf extract (*Xylocarpus granatum* J. Koenig) contains bioactive compounds with potential photoprotective properties. In this study, the extract was formulated into an oil-in-water (O/W) cream suitable for cosmetic applications.
Objective: This study aimed to determine the in vitro sun protection factor (SPF), percentage of erythema transmission (%Te), and percentage of pigmentation transmission (%Tp) of the mangrove leaf extract cream, and to evaluate its effectiveness on UVB-exposed guinea pig skin using collagen density as an anti-aging biomarker.
Methods: In vitro assessments of SPF, %Te, and %Tp were performed using UV–Vis spectrophotometry. In vivo evaluation was conducted using 20 guinea pigs to examine the effect of the cream on collagen density through histopathological analysis of UVB-exposed skin.
Results: The mangrove leaf extract cream exhibited strong photoprotective activity, with SPF values ranging from 20.68 ± 0.51 to 33.70 ± 0.67, %Te values from 3.13 ± 0.08 to 1.75 ± 0.03, and %Tp values from 4.40 ± 0.12 to 3.31 ± 0.03. Furthermore, the cream significantly increased collagen density (71.77 ± 5.27–81.02 ± 0.96) compared with the positive control (52.16 ± 2.32) ($p < 0.05$).
Conclusions: The mangrove leaf extract cream provides effective protection against UVB radiation, characterized by high-protection SPF, extra-protection %Te, and sunblock-level %Tp. The 5% formulation significantly enhanced collagen density, supporting its potential as a natural anti-aging active ingredient for photoaging prevention through improved UV protection and dermal structure.

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INTRODUCTION

Skin aging is a multifactorial physiological and pathophysiological process characterized by progressive deterioration of the skin's structural integrity and functional capacity, influenced by hormonal, metabolic, genetic, and environmental factors.[1,2] Among these contributors, sunlight exposure plays a dominant role and is estimated to account for up to 90% of clinically visible skin aging. Repetitive and prolonged exposure to ultraviolet (UV) radiation accelerates degenerative changes in the skin, a process commonly referred to as photoaging. Photoaging is associated with inflammation, hyperpigmentation, collagen degradation, wrinkle formation, dehydration, and increased skin roughness.[3,4] Although UVB represents only a small portion of the UV spectrum, it is the most biologically active wavelength in inducing epidermal and dermal damage, making it a critical factor in the development of photoaging-related alterations.[5]

Repeated exposure to UVB radiation induces the abnormal production of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and hydrogen peroxide, which collectively trigger oxidative stress within biological systems.[5–7] Elevated ROS levels activate the mitogen-activated protein kinase (MAPK) signaling cascade, subsequently enhancing the expression and phosphorylation of c-Jun and c-Fos. These proteins form the transcription factor complex activator protein-1 (AP-1), which plays a central role in UV-induced skin damage.[8,9] AP-1 suppresses the

activity of transforming growth factor-β (TGF-β), a crucial mediator of type I procollagen synthesis in the skin, thereby reducing collagen precursor expression and contributing to wrinkle formation and accelerated skin aging.[3,5,9] Moreover, AP-1 enhances the expression of several matrix metalloproteinases (MMPs), particularly MMP-1, MMP-3, and MMP-9, whose combined activity leads to extensive degradation of dermal type I and III collagen. tissue inhibitors of metalloproteinases (TIMPs) prevent collagen degradation by inhibiting matrix metalloproteinase (MMP) activity.[5,8,9] Another major transcription factor activated by UV irradiation is nuclear factor kappa-B (NF-κB), which promotes inflammatory responses and regulates the expression of MMPs such as MMP-1 and MMP-3 in human dermal fibroblasts. Thus, the activation of AP-1 and NF-κB contributes to collagen breakdown and the development of structural and functional skin alterations characteristic of photoaging.[6,10,11]

Collagen is the principal fibrillar protein within the extracellular matrix (ECM) and plays a fundamental role in maintaining the skin's mechanical strength, resilience, and elasticity. These collagen fibers are synthesized primarily by fibroblasts in the deeper layers of the skin, and adequate nutrient supply through the bloodstream is essential for optimal biomatrix renewal. Type I collagen represents the most dominant subtype, comprising approximately 80–90% of total dermal collagen. During the aging process, type I collagen undergoes progressive fragmentation, a structural alteration that compromises dermal integrity and contributes significantly to wrinkle formation.[12–14]

One of the primary strategies for preventing UV-induced photoaging is the use of sunscreen formulations capable of blocking or attenuating ultraviolet (UV) radiation. The effectiveness of a sunscreen product in protecting the skin from UV exposure is determined by its Sun Protection Factor (SPF), which indicates its ability to safeguard the skin from erythema-inducing radiation. The photoprotective potential of sunscreen agents can be further evaluated by determining the SPF value together with measurements of erythema transmission (%Te) and UV transmission (%Tp). These parameters provide insights into the extent to which a formulation absorbs or transmits ultraviolet radiation, thereby predicting its efficacy in mitigating UV-induced skin damage.[15–17]

Various sunscreen formulations have been developed to protect the skin from UV radiation, with creams being one of the most widely used forms. Cream-based preparations are preferred because they exhibit good skin penetration, spread easily across the skin surface, and provide a pleasant cooling sensation upon application.[18] Optimal dermal penetration of creams is influenced by their biphasic characteristics, which enable them to traverse the lipid bilayer membrane of the epidermis. This membrane consists of both hydrophilic and hydrophobic layers; the hydrophilic phase interacts with water and plays an essential role in maintaining skin moisture by reducing trans epidermal water loss.[19] In general, creams are categorized into two types: oil-in-water (O/W) and water-in-oil (W/O) emulsions. Among these, O/W creams are more commonly utilized in cosmetic formulations because they are easy to remove with water, non-greasy during application, and do not leave stains on clothing.[20]

A variety of antioxidant agents have been developed to reduce wrinkles and promote skin-brightening effects. These agents include arbutin, epigallocatechin-3-gallate (EGCG), kojic acid, vitamin C, and several other bioactive compounds. However, concerns regarding their long-term safety profiles remain unresolved,[4] thereby encouraging the exploration of alternative cosmetic ingredients derived from natural sources. One promising candidate is *Xylocarpus granatum*, a mangrove species traditionally used by coastal communities as a cooling topical powder for skin protection. Previous studies have reported that the fruit extract of *X. granatum* possesses an SPF value of 2.17 along with very strong antioxidant activity (IC_{50} = 4.78 ppm), whereas its leaf extract exhibits a high-protection potential with an SPF value of 35.56 and an IC_{50} of 64.57 ppm. Moreover, *X. granatum* leaf extract has been shown to effectively reduce epidermal hyperplasia in UVB-exposed guinea pig skin, further supporting its potential as a natural photoprotective and anti-photoaging agent.[21]

However, no studies have investigated the SPF value of *Xylocarpus granatum* leaf extract formulated into a cream, nor its potential as an anti-aging agent through molecular or biomarker-based approaches. Therefore, this study aimed to determine the in vitro sun protection factor (SPF), percentage of erythema transmission (%Te), and percentage of pigmentation transmission (%Tp) of the leaf extract cream, and to evaluate its effectiveness in UVB-exposed guinea pig skin by assessing collagen density as a key anti-aging biomarker. This study is expected to provide scientific contributions to the development of green cosmeceutical ingredients derived from Indonesia's coastal biodiversity, particularly those capable of preventing photoaging through enhanced photoprotection and restoration of dermal collagen density.

RESEARCH METHODS

Materials and Instruments

Materials. The materials included mangrove leaf cream formulations (*Xylocarpus granatum*) as listed in **Table 1**, 2% hydroquinone cream, Hematoxylin–Eosin (HE) reagents, 10% formalin, ketamine (100 mg/kg BW), ethanol (70%, 80%, 90%), xylol solution, and liquid paraffin.

Instruments. The instruments used in this study included a UVB irradiation device (KN-4003®), electric shaver (Gillette Goal®), digital balance (Ohaus®), optical microscope (Olympus CX42®), and rotary evaporator (Buchi R-300®).

Research Procedure

Study Design

This experimental study employed a randomized posttest-only control group design. A total of 20 guinea pigs were randomly assigned into five groups: positive control (2% hydroquinone cream + UVB), negative control (cream base + UVB), MLC-1 (mangrove leaf cream 5%), MLC-2 (mangrove leaf cream 10%), and MLC-3 (mangrove leaf cream 15%).

Extract Preparation

Mangrove leaf simplicia powder was extracted using an ultrasonication and dynamic maceration-assisted extraction technique as described by Andishmand (2023).[22] Ultrasonication was performed at a frequency of 50–60 kHz using an elmasonic device. A total of 100 g of simplicia powder was placed into a beaker and mixed with 600 mL of 80% ethanol at a ratio of 1:6 (w/v). Ultrasonication was carried out at 40 °C, with each cycle consisting of 5 minutes of sonication followed by a 5-minute pause (1 cycle = 10 minutes). The cycle was repeated twice, resulting in a total active sonication time of 10 minutes. The mixture underwent dynamic maceration for 24 hours with stirring every six hours. The mixture was then filtered to obtain the extract filtrate. The extraction process was repeated twice. All collected filtrates were concentrated using a rotary evaporator at 40 °C to obtain a viscous extract.

Determination of SPF Value of Mangrove Leaf Extract

The Sun Protection Factor (SPF) of the mangrove leaf extract–based sunscreen cream was determined *in vitro* using a UV–Vis spectrophotometry. The mangrove leaf extract cream was initially prepared at a concentration of 1000 ppm. Aliquots of 7 mL, 8 mL, 9 mL, and 10 mL were then transferred into 10 mL volumetric flasks and diluted to volume with pro analysis ethanol to obtain graded concentrations of 700 ppm, 800 ppm, 900 ppm, and 1000 ppm, respectively. Each solution was filtered using Whatman No. 1 filter paper. A total of 3 mL of each filtrate was placed into a quartz cuvette, and the absorbance was measured at wavelengths ranging from 290 to 400 nm at 5 nm intervals. All measurements were performed in triplicate.[23]

a. Determination of Sun Protection Factor (SPF)[24]

The SPF value was determined by calculating the area under the absorption curve (AUC), derived from the absorbance values measured at wavelengths ranging from 290 to 400 nm. The AUC was subsequently calculated using the following formula (1).

$$AUC = \frac{Aa + Ab}{2} \text{ x } dPa - b \quad \text{..... (1)}$$

Where:
Aa = absorbance at the wavelength a nm
Ab = absorbance at the wavelength b nm
dPa-b = the difference in wavelengths a and b

The total AUC value was calculated by summing the AUC values for each wavelength. The SPF value for each concentration was then determined using the following formula (2).

$$\Sigma AUC = L1 + L2 + L3 + L4 + L5 + L6 + L7 \ldots\ldots\ldots Ln$$

$$\text{Log SPF} = \frac{AUC}{\lambda n - \lambda 1} \quad \text{..... (2)}$$

Where:
Log SPF = AUC / λ n – λ 1 (12)
λ n = the largest wavelength (with A> 0.05 for extracts and A> 0.01 for preparations)
λ 1 = the smallest wavelength (290 nm)

b. Determination of Erythema Transmission (%)[24]

Transmittance values obtained at each wavelength were subsequently used to calculate the percentage of erythema transmission by determining the erythema transmission parameter (T·Fe). The amount of erythema flux transmitted through the sunscreen formulation (Ee) was then quantified using the following formula (3).

$$Ee = \Sigma T.Fe \quad \ldots\ldots (3)$$

The percentage of erythema transmission was calculated using the following formula (4).

$$\% \text{ Erythema Transmission} = \frac{Ee}{\Sigma Fe} \quad \ldots\ldots (4)$$

Where:
Fe = Erythema flux
T = Transmission value
Ee = ΣT. Fe = the amount of erythema flux transmitted by the extract at a wavelength of 292.5 – 317.5 nm.

c. Determination of Pigmentation Transmission (%)[24]

The percentage of pigmentation transmission was determined by first calculating the pigmentation transmission value (T·Fp). The amount of pigmentation flux transmitted through the sunscreen sample (Ep) was then obtained using the following formula (5).

$$Ep = \Sigma T.Fp \quad \ldots\ldots (5)$$

The percentage of pigmentation transmission was calculated using the following formula (6).

$$\% \text{ Pigmentation Transmission} = \frac{Ep}{\Sigma Fp} \quad \ldots\ldots (6)$$

Where:
Fp = pigmentation flux
T = transmission value
Ep = ΣT.Fp = the amount of erythema flux transmitted by the extract at a wavelength of 322.5- 372.5 nm
ΣFp = the total amount of UV light energy that causes pigmentation.

The percentage of erythema transmission (%Te) and pigmentation transmission (%Tp) are commonly used parameters for classifying sunscreen efficacy. Based on these values, sunscreen performance can be categorized as **sunblock** (%Te < 1% and %Tp 3–40%), **extra protection** (%Te 1–6% and %Tp 42–86%), **suntan** (%Te 6–12% and %Tp 45–86%), and **tanning** (%Te 10–18% and %Tp 45–86%).[25]

Table 1. Formulation of Mangrove Leaf Extract Cream

Ingredient	Concentration (%)			Function
	MLC1	MLC2	MLC3	
Mangrove leaf extract	5	10	15	Active ingredient
Triethanolamine (TEA)	4	4	4	Emulsifier
Stearic acid	8	8	8	Emulsifier
Cethyl alcohol	4	4	4	Emulsifier
Glycerin	11	11	11	Emollient
Methyl paraben	0.2	0.2	0.2	Preservative
Propyl paraben	0.2	0.2	0.2	Preservative
Distilled water	ad 100	ad 100	ad 100	Base

Preparation of Mangrove Leaf Extract Cream

The oil phase (stearic acid, cethyl alcohol, and propyl paraben) and the aqueous phase (TEA, glycerin, methyl paraben, and distilled water) were separately heated in a water bath until reaching a temperature of 55 °C. The aqueous phase was then gradually added to the oil phase while maintained on the water bath, followed by continuous stirring until a cream emulsion was formed. Both phases were subsequently triturated in a pre-heated mortar to obtain a uniform cream base. The mangrove leaf extract was dissolved in TEA at a 1:1 ratio, along with three drops of glycerin, in a separate mortar until completely solubilized. The resulting solution was incorporated into the cream base and triturated until a homogeneous formulation was obtained. The composition of the mangrove leaf extract cream formulations is presented in **Table 1.**

Collagen Density Analysis

The effectiveness of the mangrove leaf extract cream on collagen density in UVB-exposed guinea pig skin was evaluated in accordance with the approved by the Animal Ethics Committees of Universitas Udayana (Approval No.: B/83/UN14.2.9/PT.01.04/2023). Male guinea pigs aged 11–13 weeks and weighing 300–350 g were acclimatized for seven days prior to treatment. The animal housing conditions were maintained under a natural light–dark cycle. The ambient temperature of the cages was controlled at approximately 23–25 °C, and the guinea pig cages were cleaned twice weekly.[26] Hair on the dorsal area of each animal was shaved to a size of 4 × 4 cm, following the procedure described by Harahap (2022).[27] UVB exposure was administered three times per week at an intensity of 65 mJ/cm² for 65 seconds per session, resulting in a cumulative dose of 390 mJ/cm² over two weeks.[28,29] The cream base and mangrove leaf extract cream were applied twice daily at a dose of 4 mg/cm² of the guinea pig skin surface area, consisting of application 20 minutes before UVB exposure and reapplication 4 hours after irradiation, topical application was continued on non-irradiation days, as described in the study by Idana (2020).[29] Following the final UVB exposure, all animals were rested for 48 hours to eliminate the influence of acute photodamage. Euthanasia was carried out using an intraperitoneal injection of ketamine at an overdose of 100 mg/kg body weight.[30]

The preparation of histological specimens was performed through several sequential stages, including fixation, dehydration, clearing, and embedding. This preparation method was adapted from Fithria (2017) and Sabawy (2021) with several modifications. Skin biopsy samples were obtained and measured to a standardized size of 1 × 1 cm with a depth extending to the subcutaneous layer. During the fixation stage, the skin biopsy specimens were immersed in 10% phosphate-buffered formalin for 24 hours. Following fixation, tissue trimming was performed to obtain the required tissue area. The samples were then subjected to dehydration using a graded alcohol series by sequential immersion in 30%, 40%, 50%, 70%, 80%, 90%, and 96% ethanol, each concentration applied three times for 25 minutes. The clearing stage was conducted by immersing the dehydrated tissues in a clearing agent consisting of an alcohol–xylene mixture (1:1) for 30 minutes, followed by immersion in absolute xylene until the tissues became completely transparent. Embedding was initiated with four cycles of tissue infiltration using molten paraffin. Subsequently, the tissues were embedded in liquid paraffin and allowed to solidify to form paraffin blocks. The blocks were left to harden for 24 hours to facilitate sectioning. Serial sections were cut using a microtome at a thickness of 5 µm.[31,32]

Collagen density was assessed using Haematoxylin–Eosin (H&E) staining and quantitatively analyzed through digital image analysis. Each histological slide was examined under an Olympus CX42 microscope and imaged using an Optilab Pro camera at 400× magnification. Quantification of collagen density was performed using a combination of ImageJ and Adobe Photoshop software. Collagen density was expressed as the percentage area of collagen fibers, corrected by subtracting the percentage of cut-off or excluded areas.[33,34] The percentage of collagen density was calculated using the formula (7):

Collagen density (%) = Total image area (100%) – percentage of the excised (non-collagen) area …… (7)

Data Analysis

Data processing was performed using IBM SPSS Statistics version 25 with a 95% confidence level. Data normality for sample sizes < 50 was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's Test. The data were considered homogeneous when the significance value (p) > 0.05. Subsequently, a one-way Analysis of Variance (one-way ANOVA) was conducted to determine whether significant differences existed among at least two treatment groups. When the ANOVA indicated significant differences, post hoc analysis was performed using the Least Significant Difference (LSD) test to identify specific groups exhibiting statistically significant differences.[35]

RESULT AND DISCUSSION

Phytochemical screening has been carried out on mangrove leaf extract which shows the presence of several bioactive compounds, including flavonoids, tannins, saponins and steroids. Flavonoids, phenolic acids, and polyphenols are known to possess high molecular weight structures that contribute to their photoprotective properties against UV radiation.[21] Aromatic compounds contain functional groups capable of absorbing high-energy photons from UV light. These aromatic molecules possess functional groups capable of absorbing high-energy photons from UV light and subsequently releasing the absorbed energy as heat or re-emitted light at harmless wavelengths.[36,37] A study has been conducted to determine the sun protection factor (SPF) of mangrove leaf extract, demonstrating that the extract

exhibits a high level of sun protection (SPF 35.56), categorized as high protection, along with strong antioxidant activity as indicated by an IC_{50} value of 64.57 ppm.[21] These combined properties highlight the significant potential of mangrove leaf extract to be further developed as a natural sunscreen formulation capable of preventing skin damage induced by UVB radiation.

Cream Formulation of Mangrove Leaf Extract

The mangrove leaf extract cream formulation used in this study, as presented in **Table 1**, contains stearic acid, glycerin, propylene glycol, and cethyl alcohol, all of which play essential roles in maintaining stratum corneum integrity through synergistic humectant and emollient actions. Glycerin and propylene glycol act as hygroscopic humectants that mimic the function of the natural moisturizing factor (NMF), thereby enhancing water retention within the stratum corneum and reducing trans epidermal water loss (TEWL). Improved hydration contributes to enhanced skin biomechanics, including the regulation of corneodesmosome degradation, prevention of corneocyte accumulation, and preservation of epidermal layer continuity. Conversely, stearic acid and cethyl alcohol function as emollients by mimicking the intercellular lipid bilayer structure of the stratum corneum. These components fill the intercorneocyte spaces, thereby improving cellular cohesion, smoothing the skin surface, and enhancing the capacity of intercellular lipids to absorb, retain, and evenly distribute water. In addition, these emollients provide an occlusive effect that limits water evaporation from the skin surface, contributing to sustained epidermal hydration.[38]

Maintenance of stratum corneum barrier function through such a cream base formulation is particularly critical in the context of ultraviolet-induced skin aging. A well-preserved skin barrier serves as the first line of defense against UV radiation by restricting its penetration into deeper skin layers, whereas barrier dysfunction increases susceptibility to erythema and UVB-induced damage. Therefore, the use of an effective cream base not only improves dry skin conditions but also potentially mitigates UV-induced skin aging by indirectly protecting dermal structures, including collagen fibers.[39,40]

Sun Protection Factor

The sunscreen potential of the mangrove leaf extract cream was evaluated by determining its sun protection factor (SPF), erythema transmission (%Te), and pigmentation transmission (%Tp) values. The effectiveness of a sunscreen formulation is primarily reflected by its SPF value, which serves as a quantitative indicator of its photoprotective efficacy. SPF represents the degree of protection provided by a formulation against UV-induced skin damage. A higher SPF value corresponds to stronger protection, indicating an enhanced ability of the product to reduce the penetration of harmful ultraviolet radiation into the skin.[15,16]

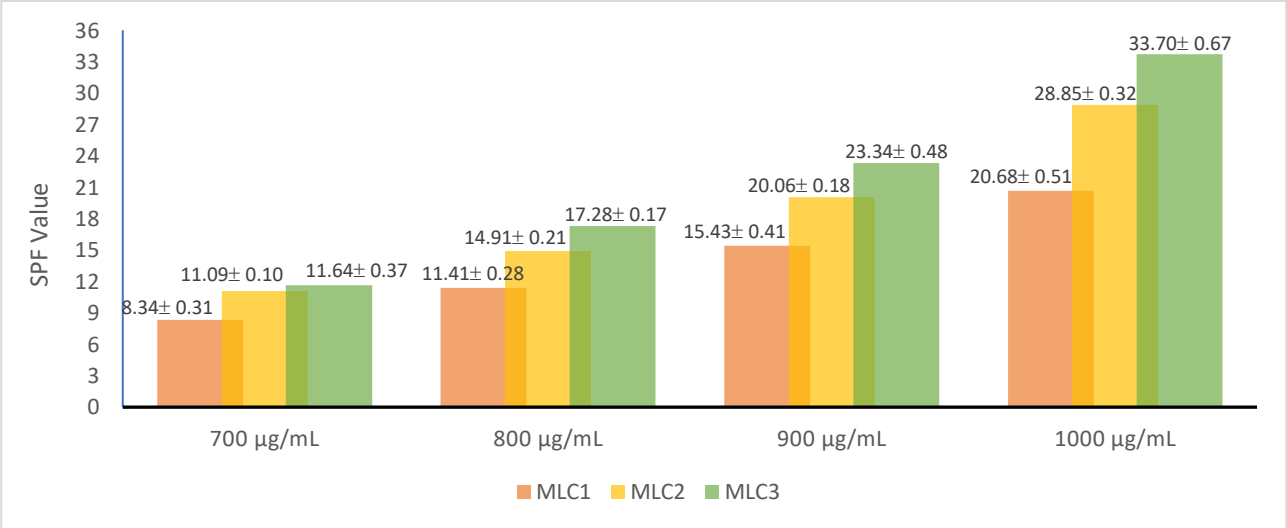

Figure 1. The Mean SPF Value of the Mangrove Leaf Extract Cream

Abbreviations: MLC1, Mangrove leaf cream 5%; MLC2, Mangrove leaf cream 10%; MLC3, Mangrove leaf cream 15%

The mean SPF values of the mangrove leaf extract creams demonstrated concentration-dependent effect, in which higher extract concentrations consistently resulted in increased SPF values. This finding is consistent with the results reported by Widyastuti et al. (2015) and Yani et al. (2023),[41,42] both of which indicated that higher extract concentrations lead to greater SPF performance. In the present study, the 15% mangrove leaf extract cream exhibited a high protection potential at a concentration of 1000 ppm, yielding a mean SPF value of 33.70 ± 0.67, as determined in

vitro using UV–Vis spectrophotometry (**Figure 1**). Sun Protection Factor (SPF) values are commonly classified into several levels of photoprotection, namely low protection (SPF 2–15), medium protection (SPF 15–30), high protection (SPF 30–50), and highest protection (SPF > 50).[43]

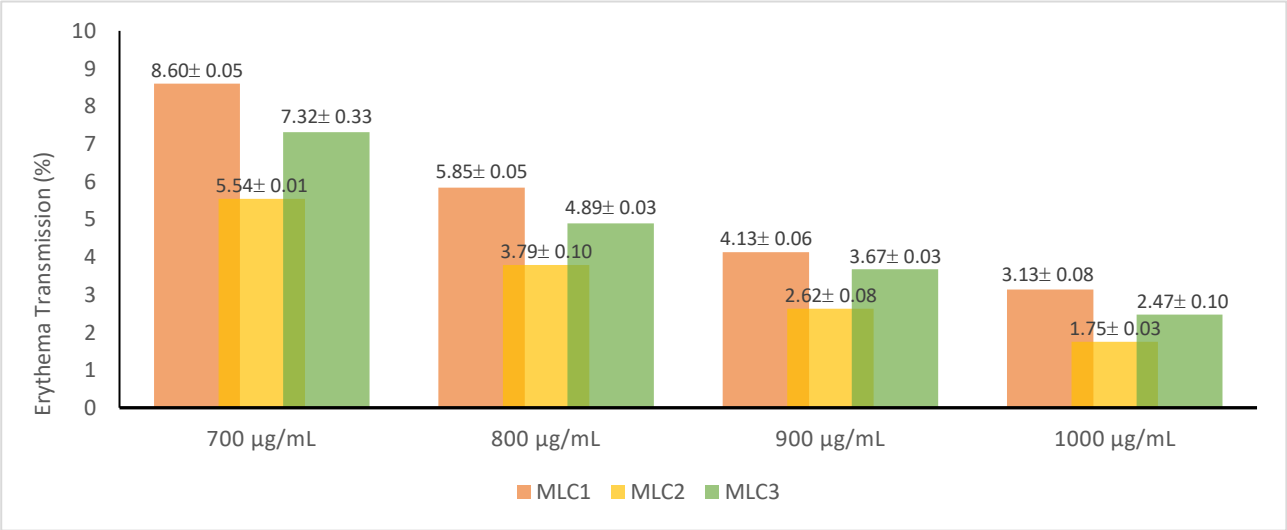

Figure 2. The Mean Percentage of Erythema Transmission of The Mangrove Leaf Extract Cream
Abbreviations: MLC1, Mangrove leaf cream 5%; MLC2, Mangrove leaf cream 10%; MLC3, Mangrove leaf cream 15%

Erythema transmission represents the fraction of UV radiation within the UVB range that passes through a sample.[17] The percentage of erythema transmission (%Te) serves as an indicator of a compound's ability to protect the skin from UVB radiation, which is responsible for inducing erythema or skin redness. As shown in **Figure 2**, the 1000 ppm concentration of MLC1, MLC2 and MLC3 into the "extra protection" category, with a %Te value of 3.13±0.08; 1.75±0.03; 2.47±0.10 (**Figure 2**). Sunscreens classified as providing extra protection can minimize erythema by absorbing more than 85% of UVB radiation, making them particularly suitable for individuals with sensitive skin.[44,45]

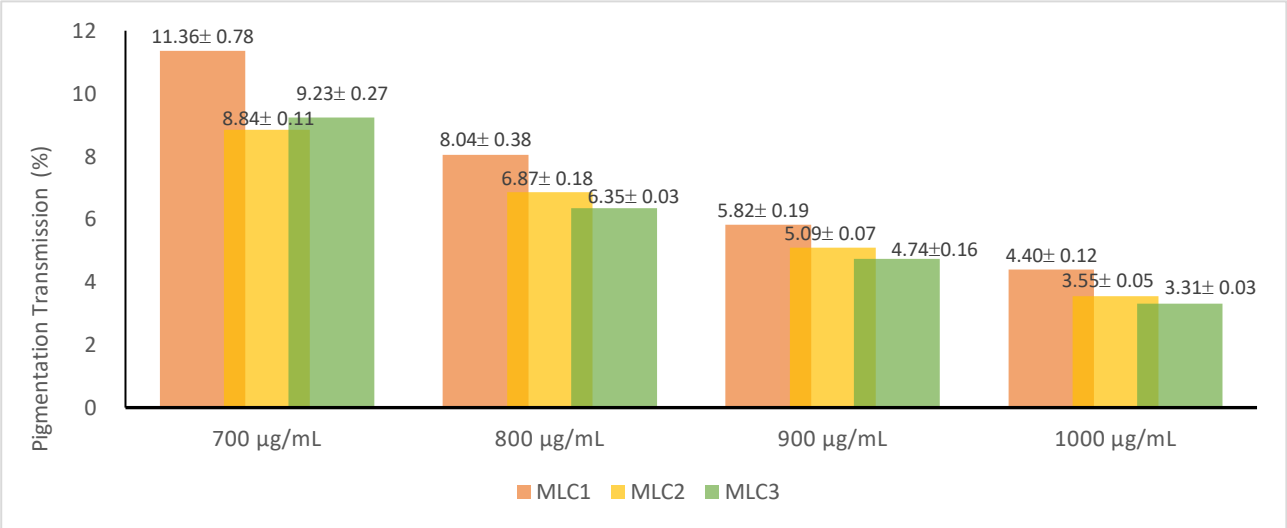

Figure 3. The Mean Percentage of Pigmentation Transmission of The Mangrove Leaf Extract Cream
Abbreviations: MLC1, Mangrove leaf cream 5%; MLC2, Mangrove leaf cream 10%; MLC3, Mangrove leaf cream 15%

Pigmentation transmission represents the amount of ultraviolet radiation within the UVA range that passes through a sample.[17] The percentage of pigmentation transmission (%Tp) describes the ability of a compound to protect the skin from UVA-induced pigmentation or skin darkening. As shown in **Figure 3**, the 1000 ppm concentration of MLC1, MLC2 and MLC3 into the "sunblock" category, with a %Tp value of 4.40±0.12; 3.55±0.05; 3.31±0.03 (**Figure 3**). The findings of this study indicate that increasing the concentration of the extract in the cream formulation resulted in a lower percentage of UV radiation transmitted to the skin that can induce pigmentation. Similar results were reported

by Sandi (2021), who demonstrated that higher extract concentrations were associated with reduced pigmentation transmission values (%Tp).[46] Sunscreens categorized as sunblock provide extensive protection, particularly for individuals with highly sensitive skin, by effectively preventing both erythema and UVA-induced pigmentation.[44,45]

Collagen Density

Collagen density in guinea pig skin exposed to UVB radiation was visualized as pink collagen fibers with purplish-blue nuclei using Hematoxylin–Eosin (HE) staining.

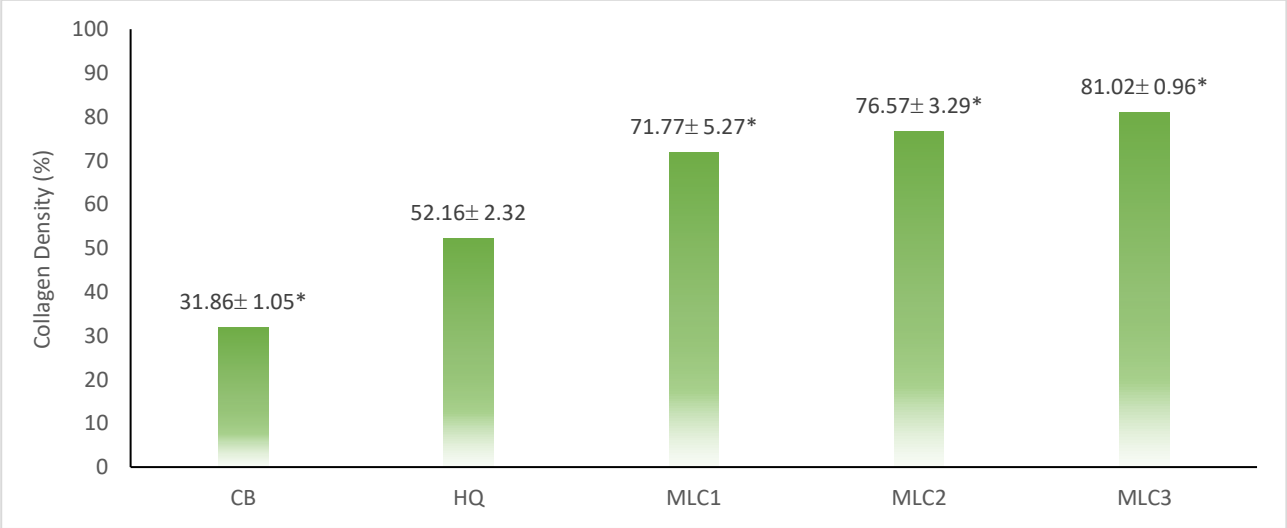

Figure 4. The Mean Value of Collagen Density at Day 14 after Radiation

Abbreviations: CB, Cream base; HQ, Hydroquinone; MLC1, Mangrove leaf cream 5%; MLC2, Mangrove leaf cream 10%; MLC3, Mangrove leaf cream 15%; (*) significant difference (p<0.05) compared with the positive control group.

Quantitative analysis showed that increasing concentrations of the mangrove leaf extract cream were associated with progressively higher collagen density (**Figure 4**). These findings are similar with the results reported by Navarro (2025), who observed a dose-dependent increase in type I procollagen following topical application of Nutroxsun® (a combination of rosemary and citrus extracts) at concentrations of 0.0005%, 0.0025%, and 0.005%. The highest concentration (0.005%) produced a statistically significant improvement compared with the negative control group ($p < 0.001$)).[47] Procollagen type I is synthesized by dermal fibroblasts and subsequently secreted into the extracellular space as a propeptide. Collagen peptidases then cleave the terminal propeptides, converting procollagen into tropocollagen. In humans, the N- and C-terminal propeptides of type I procollagen are removed by specific metalloproteinases—namely ADAMTS N-proteases and the C-protease BMP1—to generate mature type I collagen.[13]

Table 2. Collagen Density Effect of Mangrove Leaf Extract Cream

Skin assessment	Experimental group		(x̄±SD)	p-value
	Positive control group (x̄±SD)	Comparison group		
Collagen density	52.16±2.32[a]	Negative control group[b]	31.86±1.05	<0.001*
		MLC1[c]	71.77±5.27	<0.001*
		MLC2[cd]	76.57±3.29	<0.001*
		MLC3[d]	81.02±0.96	<0.001*

Notes: Mean ± standard deviation (SD); *significant difference ($p < 0.05$) compared with the positive control group; values sharing the same superscript letter indicate no statistically significant difference, while different superscript letters denote significant differences between groups.

Statistical analysis demonstrated that collagen density values were normally distributed and homogeneous ($p > 0.05$). LSD post-hoc testing revealed significant differences in collagen density between the positive control group with the negative control group, treatment groups MLC1, MLC2, and MLC3 ($p < 0.05$), indicating that the mangrove leaf extract cream effectively stimulated collagen synthesis in UVB-irradiated skin. Furthermore, comparisons between the positive control and all treatment groups (MLC1, MLC2, MLC3) also showed significant increases in collagen density ($p < 0.05$), with the mangrove extract formulations demonstrating higher collagen density than positive control (**Table 2**). The increased collagen density in the treatment groups is attributed to the bioactive constituents of the mangrove leaf

extract, which are likely to provide photoprotective effects against UVB radiation (**Figure 5**). These compounds may attenuate free radical formation and oxidative stress, thereby preventing collagen degradation and supporting fibroblast-mediated collagen synthesis.[48,49] MLC2 and MLC3 did not show a significant difference in collagen density, indicating that the response to increasing concentrations may reach a saturation point (plateau), at which additional amounts of the active compound no longer produce a significant enhancement. In other words, the concentrations used in MLC2 and MLC3 were sufficient to achieve the maximal response of the mechanistic pathways involved in collagen synthesis, thereby resulting in no statistically significant difference between these groups. These findings are consistent with those reported by Morakul (2024), who observed no significant difference in collagen synthesis between cells treated with tuna collagen peptides (TCP) at concentrations of 31.12 and 62.50 mg/mL ($p > 0.05$), whereas significant differences were detected when these concentrations were compared with a lower concentration of 15.62 mg/mL. This suggests that TCP enhances collagen synthesis in a dose-dependent manner; however, the effect tends to plateau at higher concentrations.[50]

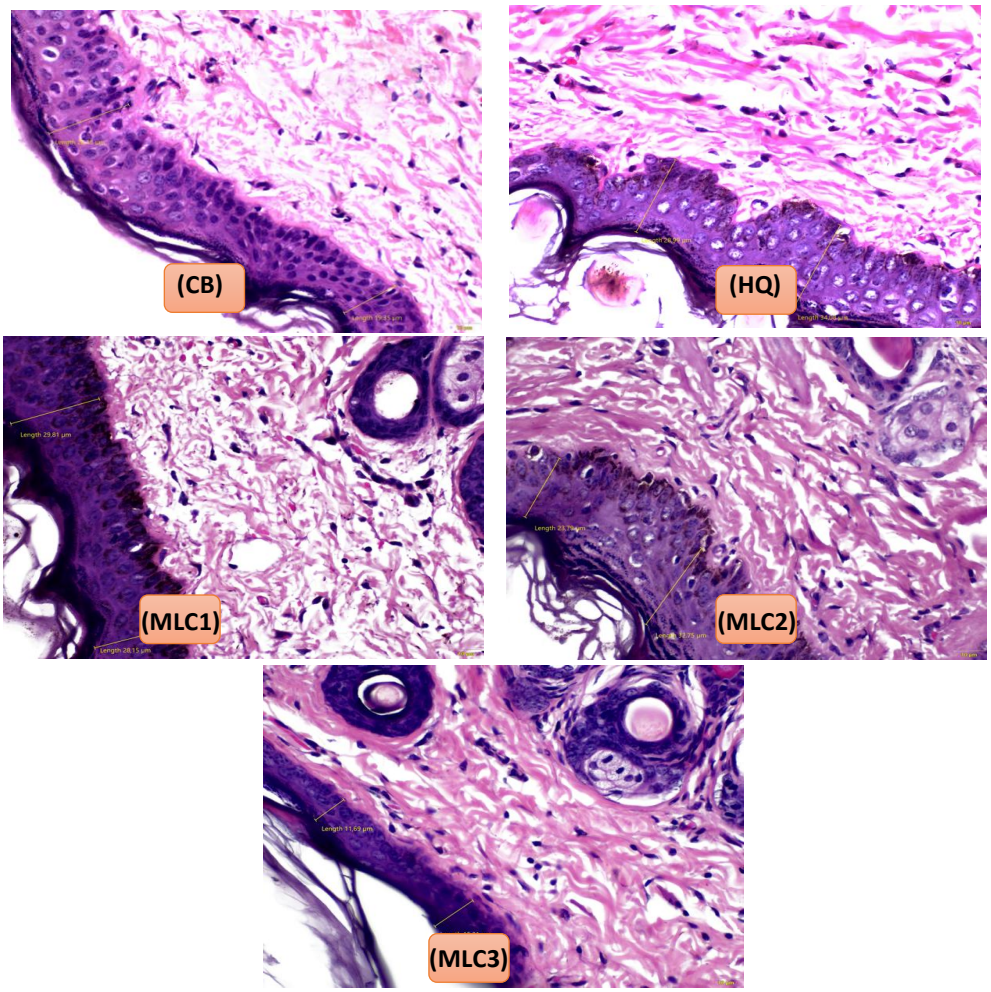

Figure 5. Histological appearance of collagen density with H&E stained.
Magnification: 400x. Abbreviations: CB, Cream base; HQ, Hydroquinone; MLC1, Mangrove leaf cream 5%; MLC2, Mangrove leaf cream 10%; MLC3, Mangrove leaf cream 15%.

Repeated exposure to UVB radiation induces abnormal production of reactive oxygen species (ROS), leading to oxidative stress that triggers inflammation, collagen degradation, premature skin aging, and even carcinogenesis.[47,51] Elevated ROS levels activate the mitogen-activated protein kinase (MAPK) signaling pathway, which subsequently increases the expression and phosphorylation of c-Jun and c-Fos, forming the transcription factor complex activator protein-1 (AP-1). AP-1 enhances the expression of matrix metalloproteinases (MMPs), particularly MMP-1, MMP-3, and MMP-9, whose combined activity of these MMPs promotes degradation of dermal collagen types I and III.[8,9] In addition, AP-1 suppresses the activity of transforming growth factor-β (TGF-β), a cytokine critical for procollagen synthesis, thereby promoting wrinkle formation and accelerating photoaging.[3,5,9] UVB irradiation also activates the NF-κB signaling

pathway, stimulating the transcription of pro-inflammatory cytokines such as TNF-α, COX-2, IL-1, and IL-6. These cytokines further promote the up regulation of MMP expression, resulting in enhanced collagen degradation.[52,53]

The use of hydroquinone as a positive control in this study was based on the potential of hydroquinone and its derivatives as antioxidant agents, one of whose mechanisms involves scavenging superoxide anions (O_2^-) generated by the hypoxanthine or xanthine oxidase system. The antioxidant activity of hydroquinone has also been extensively evaluated in vitro using DPPH, ABTS, and FRAP assays.[54,55] In addition, hydroquinone has been shown to reduce interleukin-1β (IL-1β)–induced prostaglandin E_2 (PGE_2) secretion through inhibition of cyclooxygenase (COX) activity. Hydroquinone is further reported to suppress the production of pro-inflammatory mediators, including TNF-α, IL-1β, IL-6, nitric oxide (NO), and reactive oxygen species (ROS), in monocytes, macrophages, and lymphocytes.[55]

The findings of this study are supported by previous research demonstrating that flavonoids, tannins, and saponins can effectively reduce oxidative stress in animal models.[56] Flavonoids can donate electrons or hydrogen atoms from free hydroxyl groups to neutralize free radicals, forming more stable and non-toxic flavonoid radicals.[57] Additionally, flavonoids exhibit anti-photoaging activity by suppressing MMP activity through two mechanisms: direct inhibition of MMPs and induction of tissue inhibitors of metalloproteinases (TIMPs). [58]Tannins in the mangrove leaf ethanolic extract act by donating hydrogen atoms to free radicals, converting reactive radical species into stable non-radical molecules through oxidation-reduction reactions.[59,60] The saponin content acts by scavenging superoxide radicals through the formation of hyperoxide intermediates, thereby preventing biomolecular damage caused by free radicals.[59] The steroid content is capable of suppressing NF-κB expression, thereby reducing the production of pro-inflammatory cytokines such as IL-6, IL-12, TNF-α, and COX, as well as inhibiting the expression of matrix metalloproteinases (MMPs).[61,62]

Limitation

This study presents several limitations that warrant consideration. The formulated mangrove leaf extract cream exhibited a dark green coloration, which may reduce its aesthetic acceptability when applied to the skin. Therefore, further reformulation is required to obtain preparation with more acceptable visual characteristics. In addition, the SPF value in this study was assessed solely through *in vitro* testing, and thus its *in vivo* photoprotective efficacy has not yet been established. Moreover, advanced molecular analyses are necessary to elucidate the mechanistic effects of the mangrove leaf extract cream on UVB-exposed guinea pig skin, particularly regarding its potential role in modulating collagen synthesis, antioxidant pathways, and cellular responses to UVB-induced oxidative stress.

CONCLUSION

The mangrove leaf extract cream contains bioactive compounds with the potential to provide effective protection against UVB radiation, as evidenced by SPF values within the high protection category, %Te corresponding to extra protection, and %Tp meeting the criteria for sunblock. The 5% cream formulation was shown to effectively enhance collagen density compared with the cream base and demonstrated significantly greater activity than the positive control. Overall, these findings highlight the potential of *Xylocarpus granatum* leaf extract as a promising active ingredient for the development of natural anti-aging cosmeceuticals, particularly for preventing photoaging through improved UV protection and preservation of dermal structural integrity.

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GENERATIVE AI DISCLOSURE STATEMENT

Generative AI assistance (GPT-5.2; OpenAI, 2025) was used to enhance the structural clarity and English phrasing of this paper. All AI-assisted drafts were critically evaluated and corrected by the authors. The researchers hold total responsibility for the integrity of the data and the conclusions presented herein.

AUTHOR CONTRIBUTION STATEMENT

I Gusti Agung Ayu Kusuma Wardani: Supervision, Conceptualization, Methodology, Investigation, Writing - Original Draft, Funding acquisition; **Ketut Agus Adrianta**: Validation, Formal analysis, Writing - Review & Editing; **Ni Nyoman Wahyu Udayani**: Visualization, Writing - Review & Editing; **Meriana Barreto Amaral:** Visualization, Writing - Review & Editing; **Ni Made Dharma Shantini Suena**: Resources, Writing - Review & Editing.

CONFLICT OF INTEREST DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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