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Anti-inflammatory and Anti-melanogenic Effects of *Xylocarpus granatum* J. Koenig Leaf-Extract Cream on UVB Radiation-Induced Sunburn in Guinea Pigs

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ABSTRACT

Xylocarpus granatum J. Koenig is a medical mangrove predominantly found in tropical and subtropical coastal regions, including Indonesia. The plant contains bioactive compounds such as flavonoids, tannins, saponins and steroids, which exhibit antioxidant, anti-inflammatory and anti-melanogenic effects. This study aims to determine the anti-inflammatory and anti-melanogenesis effects of *X. granatum* leaf ethanol-extract. The compounds of *X. granatum* leaf ethanol-extract were identified by using gas chromatography-mass spectrometry. The potential of the extract as a sunscreen was evaluated through the analysis of sun protection factor (SPF) value. The antioxidant activity of the extract was assessed using the DPPH method. The anti-inflammatory and anti-melanogenic effects of cream were evaluated through histopathological analysis of epidermal thickness and melanin content in ultraviolet B (UVB)-exposed guinea pig's skin. The *X. granatum* leaf extract was found to contain 16 compounds, of which 11 compounds were identified as antioxidant, anti-inflammatory, and/or anti-melanogenic. The *X. granatum* leaf extract exhibited a high level of sun protection with an SPF value of 35.56. Additionally, the extract displayed strong antioxidant activity, as indicated by an IC50 value of 64.57 ppm. The XGL 15% cream was more effective in reducing epithelial thickness than HQN. Additionally, it was equally effective in reducing melanin content compared to HQN. This study concluded that *X. granatum* leaf extract exhibit as a potential source for the development of health-related products, particularly those involving antioxidant, anti-inflammatory and anti-melanogenic properties.

Keywords: anti-inflammatory, anti-melanogenic, antioxidant, cream, *X. granatum* leaf

INTRODUCTION

Ultraviolet (UV) radiation is a known factor that contributes to skin damage by generating excessive reactive oxygen species (ROS) (Ferreira *et al*., 2020; Hong *et al*., 2020; Peng *et al*., 2020; Pratama *et al*., 2020). UV radiation consists of UVA (320-400 nm), which can penetrate the dermis, UVB (290-320 nm), causing sunburn effects on the superficial layers of the skin (epidermis), and UVC (100-290 nm), which is fully absorbed by the ozone layer and does not reach the Earth's surface (Panich *et al*., 2016; Pratama *et al*., 2020; Young *et al*., 2017). UVB is considered 1,000-10,000 times more carcinogenic than UVA (Cruz *et al*., 2020; Pratama *et al*., 2020). Acute exposure to UVB induces sunburn, hyperpigmentation, and hyperplasia (Acevedo *et al*., 2014; Ferreira *et al*., 2020).

Sunburn is an acute inflammatory reaction of the skin characterized by erythema, pain, edema, and heat, with the severity being proportional to the duration and intensity of UV exposure (D'Orazio, 2013; Guan *et al*., 2021). Inflammation is

a protective response of the immune system triggered by various harmful stimuli, including pathogens, toxins, cellular damage, or radiation (Sasadara *et al*., 2021).

UV radiation induces the proliferation of keratinocytes, which subsequently leads to the accumulation of epidermal keratinocytes. This process ultimately causes an increase in epidermal thickness and the development of skin edema (Martinez *et al*., 2018). In an inflammatory condition, the body eliminates harmful stimuli and initiates recovery. Inflammation modulates collagen synthesis, and the degree of inflammation is positively correlated with epidermal tissue thickening (Martinez *et al*., 2018; Wang *et al*., 2020). UV radiation exposure leads to DNA damage in keratinocyte cells, triggering the process of melanogenesis (Lo, 2014; Nishi *et al*., 2020). This process is divided into two pathways depending on the types of melanin produced: eumelanin and pheomelanin (Kaminski *et al*., 2022; Kumari *et al*., 2018; Serre *et al*., 2018). Melanin in the skin absorbs UV radiation, protecting the keratinocyte nucleus from UV-induced damage (Frank, 2021). However, excessive melanin production can lead to dermatological issues such as freckles, solar lentigines (age spots), melasma, cancer, and post-inflammatory melanoderma (Pillaiyar *et al*., 2017).

Hayley (2021) on the frequency of sunburn occurrence found that out of 5.438 survey results, 4,883 respondents (89.9%) reported having experienced sunburn (Hayley *et al*., 2021). According to a national data analysis in the United States in 2013, an estimated 33,826 emergency room visits were attributed to sunburn cases, costing USD 11.2 million (Bais *et al*., 2018). Sunscreen is one of the options that can protect the skin from damage caused by UV radiation. Several chemical compounds used as sunscreen include octocrylene, octyl methoxycinnamate, oxybenzone, titanium dioxide, and zinc oxide. However, the chemical compounds used in sunscreens have the potential to cause side effects such as skin irritation, allergies, and even carcinogenesis (Dinardo & Downs, 2018; Moolla, 2022). Traditional medicine is primarily trusted by 80% of the population in Asia and African countries (Dorai, 2012). Alternative and complementary medicine, including traditional medicine, offer cost-effective solutions with moderate efficacy and minimal toxicity (Fan *et al*., 2015; Sharifi *et al*., 2021). Active compounds from plants have the potential to protect against UV radiation to the skin through their anti-oxidant, anti-inflammatory, and anti-melanogenic properties (Darmadi *et al*., 2021; Feng *et al*., 2014; Swallah *et al*., 2020; L. Yang *et al*., 2020).

Xylocarpus granatum J. Koenig, a medicinal mangrove primarily found in tropical and subtropical coastal regions, including Indonesia, possesses a rich diversity of secondary metabolites, including flavonoid, saponin, tannin, terpenoid, alkaloid, anthraquinone, and cardiac glycoside (Islam *et al*., 2019; Polyium, 2020; Shi *et al*., 2017; Tomizawa *et al*., 2017). Traditionally, this plant has been used for various purposes, such as treating inflammation, dysentery, cholera, fever, malaria, viral infections, and abdominal problems (Dey *et al*., 2021). Flavonoids and tannins have been reported to protect the skin from oxidative stress-induced damage (Feng *et al*., 2014; Swallah *et al*., 2020). Moreover, flavonoids, tannins, and saponins have demonstrated anti-melanogenic properties (Gazali *et al*., 2014; Trisnawati *et al*., 2019).

Traditionally, the communities in Teluk Balikpapan, East Kalimantan, Indonesia have utilized mangrove fruit seeds as a cooling powder for gardening and aquaculture activities (Ma'ruf, 2022). Similarly, in Central Java, the local community has recognized the moisturizing properties of mangrove fruits for skin care (Pringgenies *et al*., 2021). Other studies have suggested that *Xylocarpus granatum* (XG) extract has the potential as an anti-inflammatory and antioxidant agent (Islam *et al*., 2019). Preliminary research has shown that the fruit and leaf extract of X. *granatum* possesses sun protection factor (SPF) values of 2.17 and 35.56, respectively. However, a research gap exists regarding X. *granatum* leaf extract for sunburn protection. Therefore, this current study aims to determine the anti-inflammatory and anti-melanogenic effect of *Xylocarpus granatum* leaf ethanol-extract cream, addressing the need for natural remedies to safeguard against sunburn. The selection of cream formulations is based on the cream base, which plays a crucial role in topical drug delivery to enhance drug penetration into skin tissues. Oil-in-water creams contain a high water content, which provides a moisturizing effect on the skin and penetrates active ingredients, resulting in an immediate effect after application (Brown *et al*., 2018; Simões *et al*., 2018). Therefore, oil-in-water cream can be considered suitable for sunscreen formulation (Hasniar *et al*., 2015). Prompted by the previous data, this current study identified chemical compounds in X. *granatum* leaf extract followed by formulation in a topical preparation.

Additionally, we assessed the extract's antioxidant activity through in vitro testing using the DPPH assay. Significantly, we investigated the potential in vivo anti-inflammatory and anti-melanogenic effects of the X. *granatum* extract cream by evaluating parameters such as epidermal thickening and melanin contents.

MATERIALS AND METHODS

X. granatum material and extract preparation

Fresh X. *granatum* samples were obtained from Taman Hutan Raya Ngurah Rai, Badung, Bali. The samples were cleaned of impurities and washed with running water. Subsequently, the samples were chopped to reduce their size. The leaves were dried in an oven at a temperature of 40°C for 3-5 days until a constant weight was achieved. The extraction of bioactive compounds from X. *granatum* was carried out in several steps. X. *granatum* leaf powder (150 g) was dissolved in 80% ethanol with a ratio of 1:10 (simplicia:solvent) at room temperature. The extraction process was conducted using an ultrasonic apparatus for 10 minutes at a temperature of 40°C. This step was repeated three times, followed by maceration for two days. The collected filtrate evaporated at 40°C until a concentrated extract was obtained. The crude extract yield was determined using the formula created by Susilowati and Purwati (2021).

$$\text{Rendement (\%)} = \frac{\text{weight extract after extraction}}{\text{weight simplicia before extraction}} \times 100$$

Phytochemical analysis

A total of 6 g of X. *granatum* leaf extract was dissolved in 60 mL of 80% ethanol. The resulting diluted extract was subjected to qualitative analysis to determine flavonoids, alkaloids, saponins, tannins, phenols, and steroids or terpenoids using established protocols (Agada *et al*., 2020; Morsy, 2014).

GC–MS analysis

The crude extracts of X. *granatum* were subjected to GC-MS analysis for compound detection. The GC-MS analysis was performed using an Agilent 8860 GC System coupled with an Agilent 5977B GC/MSD equipped with an HP-5MS UI column (30∗0.25mM, 0.25 μM). The samples were injected 1uL in splitless mode. The initial temperature was set at 70°C for 5 min, followed by a temperature ramp of 10°C per min up to 270°C for 15 min. The total run time was 40 min, and the injection port temperature was maintained at 280°C. The helium flow rate was set to 3 mL/min, and the ionization voltage was set to 70 eV. Mass spectra (MS) scans were performed in the range of 50 to 300 m/z. The identification of each compound was based on comparing the mass spectra (MS) with standard reference databases such as NIST17.L.

Determination of SPF from XG leaf extract

The sun protection factor (SPF) of the XG leaf extract was determined according to research conducted by Indrisari *et al*. (2021). The SPF value of the extract was assessed by creating a concentration gradient ranging from 100 to 1,300 ppm and measuring it within the wavelength range of 290-400 nm at 5 nm intervals. At a concentration of 1,300 ppm, the extract exhibited an SPF value categorized as high protection, equivalent to 0.13% concentration extract.

Antioxidant activity of XG leaf extract

The DPPH method was used to evaluate the radical scavenging potential of the X. *granatum* leaf extract. Initially, 2 ml of a 40 ppm DPPH was mixed with 2 mL of the XG leaf extract at various concentrations (40, 60, 80, 100, 120, 140, and 160 ppm). The solution was maintained in a dark environment at room temperature for 30 minutes. The measurement of the optical density (OD) of the solutions was done using a spectrophotometer at 516 nm. The IC50 value was determined through a calibration curve, plotting the associated scavenging effect of the X. *granatum* leaf extract. The radical scavenging activity was calculated using the subsequent formula:

$$\text{Scavenging (\%)} = \frac{(A_1 - A_2)}{A_1} \times 100 \quad \text{(Vikas } et\ al.\text{, 2017)}$$

A_1 = negative control absorbance; A_2 = sample absorbance

Formulation of cream

The extract was distributed into a cream formulation using an oil-in-water (O/W) emulsion system (Table I). This formula was adapted and modified from previous research conducted by Wardani *et al*. (2023). The water-soluble components (TEA, glycerin, methylparaben, and aquadest) were heated at a temperature between 70°C and 75°C. The oil-soluble components (stearic acid, propylparaben, and cetyl alcohol) were heated to the same temperature range.

Table I: The formulation of XG leaf-extract cream

Ingredients	XGL 5% (%)	XGL 10% (%)	XGL 15% (%)	Function
X.G leaf extract	5	10	15	Active ingredient
TEA	4	4	4	Emulsifier
Stearic Acid	8	8	8	Emulsifier
Cetyl alcohol	4	4	4	Emulsifier
Glycerin	11	11	11	Emollient, humectant
Methylparaben	0.2	0.2	0.2	Preservative
Propylparaben	0.02	0.02	0.02	Preservative
Aquadest	ad 100	ad 100	ad 100	Vehicle

Abbreviations: XGL 5%, *Xylocapus granatum* leaf-extract cream 5% concentration; XGL 10%, *Xylocapus granatum* leaf-extract cream 10% concentration; XGL 15%, *Xylocapus granatum* leaf-extract cream 15% concentration

Once dissolved, the aqueous phase gradually added to the oil phase while continuously stirring to ensure homogeneity and the formation of a creamy consistency. The X. *granatum* leaf extract was added to the cream mass and stirred until fully dispersed.

Animal experiments

Fifteen male guinea pigs, aged between 11-13 weeks, were obtained from the Pharmacology Laboratory, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Bali, Indonesia. The guinea pigs were housed in a controlled environment with a 12-hour light/dark cycle at room temperature. All guinea pigs were fed a standard pellet diet and provided with water ad libitum.

Animal model

The animal experiment conducted in this study received approval from the Institutional Ethical Committee of the Faculty of Veterinary Medicine, Udayana University (approval number: No. B/83/UN14.2.9/PT.01.04/2023). Fifteen guinea pigs were randomly assigned to five groups: the CBG groups administered the base cream 20 minutes before and 4 hours after UVB exposure at a dose of 65 mJ/cm^2; the HQN group administered the hydroquinone cream under the same conditions; and the treatment groups administered 5%, 10%, and 15% extract creams (respectively) before and after UVB exposure with the same dose. The creams were applied to a shaved area of the guinea pig's skin, covering a total area of 3 cm^2 at a dose of 4mg/cm^2 (Indrayani *et al*., 2020). Both the control and study groups were exposed to UVB light for 65 seconds at a constant dose of 65 mJ/cm^2. The intervention lasted two weeks after an initial adaptation period, with three exposures per week and a total dose of 390 mJ/cm^2. To rule out acute irradiation effects, all guinea pigs were given a 48-hour resting period after the final exposure (Idana *et al*., 2022).

Histological analysis

The histological preparation process consisted of four stages: fixation, dehydration, clearing, and embedding. Each biopsy skin tissue had a 1-cm length and 1-cm width. It has a depth of up to the subcutaneous layer. The fixation stage involved soaking the biopsied skin in 10% phosphate-buffered formalin for 24 hours, followed by trimming the tissue to be taken. Additionally, the tissue was dehydrated using graded alcohol. The tissue was soaked successively in 30%, 40%, 50%, 70%, 80%, 90%, and 96% alcohol, each for 25 minutes, three times per concentration. The tissue was then cleared by immersing it in a clearing agent made of alcohol and xylene in a 1:1 ratio for 30 minutes and dipping it in pure xylene until it became transparent. The embedding stage started with the infiltration process using pure paraffin four times. Then, the tissue was embedded in liquid paraffin and allowed to form a block. This process took one day to be easily sliced with a microtome. The microtome was used to cut the tissue into 5-micrometer-thick sections, and the 5th, 10th, and 15th slices were selected for further attachment to glass objects. The glass objects were coated with adhesive and painted with Masson-Fontana. The visualization of the skin tissue's microstructure, including parameters such as epidermal thickness and melanin contents, was achieved through Hematoxylin and Eosin (H&E) staining. The prepared skin tissue slides were examined under an Olympus CX42 microscope, and images were captured using an Optilab Pro camera at a magnification level of 400x.

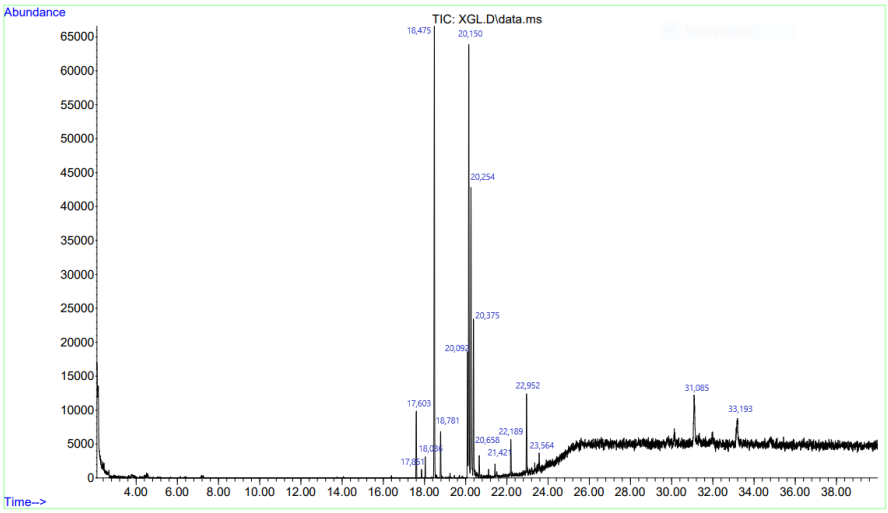

Figure 1. The chromatogram of the XG leaf extract. The peak with the highest intensity at a retention time of 18.475 corresponds to the identification of Hexadecanoic acid, methyl ester as the abundant compound in the XG leaf extract.

Measurement of melanin content

The quantity of melanin was determined by counting the number of cells in melanin granules within their cytoplasm. This calculation was based on observations of five fields of view from tissue sample sections. The number of cells was then counted.

Data processing and statistical analysis

The mean values of epithelial thickness and melanin content from each group were statistically analyzed. The analysis began with a normality test followed by a homogeneity test using Levene's test. Quantitative data were presented as mean±SD. Analysis of variance (ANOVA) was applied to identify significant differences between groups, followed by the post hoc least significant difference (LSD) test for further comparison. SPSS (Version 26) facilitated all statistical analyses in this study.

RESULTS AND DISCUSSION

Extract characterization

The X. *granatum* leaf extract yield was obtained at 21.94±0.01%. Phytochemical screening of the XG leaf extract revealed the presence of secondary metabolites such as flavonoids, tannins, saponins, and steroids, as confirmed by color reactions. These active compounds demonstrate photoprotective activity, supporting the utilization of X. *granatum* leaf extract potentially having a high protective Sun Protection Factor (SPF). The aromatic compounds in flavonoids and tannins can absorb high-energy photons from UV radiation, releasing the energy in heat or harmless light wavelengths (Mapoung *et al*., 2020; Mota *et al*., 2020).

GC-MS analysis

The bioactive compounds present in the XG leaf extract were identified using GC-MS analysis. The chromatogram displayed consistent peaks at various retention times, indicating the presence of multiple compounds (Figure 1).

The analysis revealed the presence of sixteen peaks, indicating the presence of phytochemical constituents. The most predominant compounds in terms of abundance were 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (22.69%), Hexadecanoic acid, methyl ester (22.49%) and phytol (17.26%). Detailed information on the compound names, synonyms, molecular formulas, molecular weights (MW), retention times (RT), areas under the curve (AUC), and pharmacological activities, sorted in descending order of AUC (Table II). Additionally, the XG leaf extract exhibited the presence of phenolics, steroids, fatty acids, and other compounds. The XG leaf extract primarily contained 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- as the most abundant compound, followed by hexadecanoic acid, methyl ester, and phytol. Chromatography analysis of various studies showed that 11 of 16 compounds of XG leaf extract had antioxidant, anti-inflammatory, and/or anti-melanogenic activities (Table II).

Table II. The analysis of bioactive compounds found in the XG leaf extract using GC-MS

No	Name of compound	Synonym	Molecular formula	MW	RT	AUC (%)	Pharmacological activities
1	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Linolenic acid, methyl ester:	$C_{19}H_{32}O_2$	149.2	20.15	22.69	Antioxidant (Jalalvand *et al*., 2019) Anti-inflammatory (Moreno *et al*., 2018) Anti-melanogenic (Teng *et al*., 2020)
2	Hexadecanoic acid, methyl ester	Metil palmitat	$C_{17}H_{34}O_2$	270.4	18.475	22.49	Anti-inflammatory, Antioxidant (Sharawy *et al*., 2013)
3	Phytol	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, (2E,7R,11R)	$C_{20}H_{40}O$	135.0	20.254	17.26	Anti-inflammatory, Antioxidant (Srinivasan *et al*., 2017; Swamy *et al*., 2015) Anti-melanogenic (Ko *et al*., 2018)
4	Methyl 8-methyl-nonanoate	Isocapric acid methyl ester	$C_{11}H_{22}O_2$	255.2	20.375	8.18	No activity
5	Chloroacetic acid, dodec-9-ynyl ester	9-Dodecynyl chloroacetate	$C_{14}H_{23}ClO_2$	149.2	20.092	5.96	Anti-inflammatory (Mohammad *et al*., 2019) Antioxidant (Tareq *et al*., 2023)
6	Arsenous acid, tris (trimethylsilyl) ester	Tris (trimethylsiloxy) arsin	$C_9H_{27}AsO_3Si_3$	281.1	31.085	5.76	No activity
7	Cyclotrisiloxane, hexamethyl-	Hexamethylcyclotrisiloxane	$C_6H_{18}O_3Si_3$	281.1	33.193	4.34	Anti-melanogenic (Ha *et al*., 2021)
8	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	[2,6,6-trimethyl-5-(3-methylbut-2-enyl)cyclohex-2-en-1-yl]methanol	$C_{15}H_{26}O$	281.0	22.952	3.63	No activity
9	Neophytadiene	7,11,15-trimethyl-3-methylidenehexadec-1-ene	$C_{20}H_{38}$	123.2	17.603	2.92	Anti-inflammatory (Prasathkumar *et al*., 2022) Anti-melanogenic (Younis *et al*., 2022)
10	Dodecanoic acid	Lauric acid	$C_{12}H_{24}O_2$	185.1	18.781	2.04	Anti-inflammatory (Ahmad *et al*., 2015; Dubo *et al*., 2019) Antioxidant (Miya *et al*., 2023)
11	Cyclopentanecarboxamide, N-(4-fluorophenyl)-	N-[4-(4-fluorophenyl)-1,3-thiazol-2-yl]cyclopentanecarboxamide	$C_{12}H_{14}FNO$	281.1	22.189	1.66	No activity
12	n-Decanoic acid	Decanoic acid, Capric acid	$C_{10}H_{20}O_2$	207.1	20.658	0.78	Anti-inflammatory, Antioxidant (Sharma *et al*., 2023)

Continue of Table II

No	Name of compound	Synonym	Molecular formula	MW	RT	AUC (%)	Pharmacological activities
13	4-Methyl-2,4-bis (phydroxyphenyl) pent-1-ene, 2TMS derivative		$C_{24}H_{36}O_2Si_2$	281.2	23.564	0.76	Antioxidant (Wiraswati *et al*., 2023)
14	6-Octen-1-ol, 3,7-dimethyl-, propanoate	Citronellyl propionate	$C_{13}H_{24}O_2$	123.2	18.036	0.6	Antioxidant (Ali *et al*., 2022)
15	2-Ethenoxy-1,7,7-Trimethylbicyclo (2.2.1) heptane	bornylvinylether	$C_{12}H_{20}O$	281.1	21.421	0.41	No activity
16	Furan, 2-hexyl-	Furanone (2-hexylfuran)	$C_{10}H_{16}O$	95.1	17.851	0.25	Anti-inflammatory, Antioxidant (Rashad *et al*., 2015)

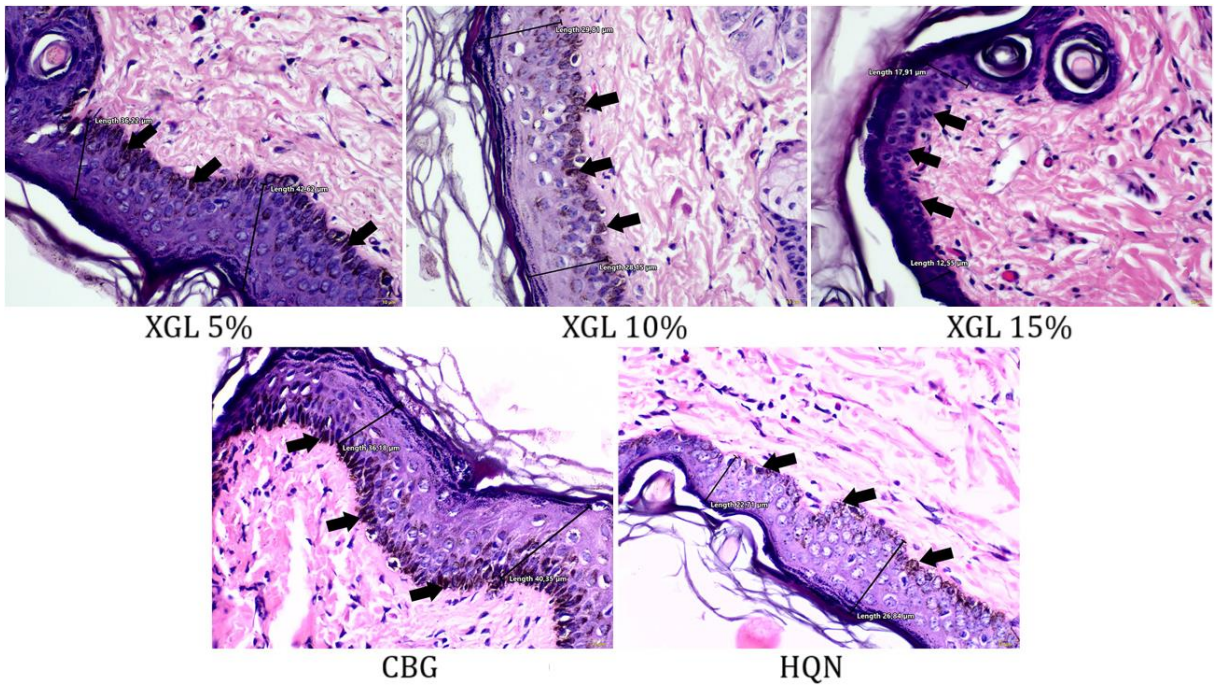

Figure 2. Histological appearance of epidermal thickness and melanin with H&E stained. Magnification: 400x. Abbreviations: CBG, Cream base group; HQN, Hydroquinone cream; XGL 5%, *X. granatum* leaf-extract cream 5% concentration; XGL 10%, *X. granatum* leaf-extract cream 10% concentration; XGL 15%, *X. granatum* leaf-extract cream 15% concentration.

SPF analysis

The leaf extract of X. *granatum* exhibited an SPF value of 35.56 (high protection), while the fruit extract showed a value of 2.17. According to the European Commission (EC) classification in 2006, SPF values fell into different categories: low protection (SPF 6-10), moderate protection (SPF 15-25), high protection (SPF 30-50), and very high protection (SPF>50) (Communities, 2006). According to studies conducted by Widyastuti *et al*. (2015) and Yani *et al*. (2023), the SPF value increases with a higher concentration of the extract. A substance is considered to have sun-protective properties if its SPF value is >2 (Indrisari

et al., 2021). Sun Protection Factor (SPF) values are commonly used to indicate the efficacy of sunscreen products (Mansuri *et al*., 2021).

In vitro antioxidant activity

The X. *granatum* leaf extract demonstrates strong antioxidant activity, as evidenced by its IC_{50} value of 64.57 ppm. A sample is considered to possess extreme antioxidant activity if its IC_{50} value is below 50 ppm, strong antioxidant (50-100 ppm), medium antioxidant if its value is 101-150 ppm), and weak antioxidant if its value is >150 ppm (Blois, 1958; Kuspradini *et al*., 2018).

Anti-inflammatory effect of XG leaf cream

The results of histopathological analysis regarding UVB-induced inflammation in guinea pigs, specifically focusing on epidermal thickness (Figure 2). The LSD analysis showed no significant difference in epidermal thickness between XGL 10% and HQN, with a *p*-value of 0.165 (p>0.005). However, XGL 15% exhibited a significant difference from HQN, with a *p*-value of 0.001. The mean value of epidermal thickness in XGL 15% (14.23±0.95) was lower than that of HQN (24.25±1.62), CBG (39.91±1.84), XGL 5% (33.29±5.66), and XGL 10% (27.72±1.12), (Figure 3) (Table III). It is indicated that the topical application of XGL 15% provides better protection in reducing epithelial thickness caused by UVB radiation compared to the other groups examined in this study.

Acute exposure to UVB radiation is associated with various skin manifestations, including sunburn, hyperpigmentation, and hyperkeratosis (Acevedo *et al*., 2014; Coelho, 2016; Ferreira *et al*., 2020). UVB radiation triggers an inflammatory response by inducing the release of cytokines, neuroactive substances, and vasoactive mediators, which can lead to hyperkeratosis. When the UVB dose exceeds the threshold for cellular damage, keratinocytes activate the apoptotic pathway, resulting in cell death, commonly referred to as sunburn cells (J. D'Orazio *et al*., 2013). Several growth factors and cytokines, such as EGF, TNF, and IFNy, can induce the expression of keratins K6, K16, and K17, which are involved in hyperkeratosis (Komine, 2018).

Hyperkeratosis characterized by epidermal thickening is the inflammatory response triggered by UVB. The increased cell division following UV exposure leads to the accumulation of epidermal keratinocytes, contributing to the thickening of the epidermis. Following extensive damage, cells undergo cell cycle arrest, activate DNA repair mechanisms, and initiate apoptosis. However, the damage response signals diminish several hours after exposure to UV radiation. This leads to vigorous proliferation of keratinocytes in the epidermis, mediated by various epidermal growth factors. The increased proliferation results in the accumulation of epidermal keratinocytes and subsequent thickening of the epidermis (D'Orazio *et al*., 2013; Sasadara *et al*., 2021).

UV radiation may damage skin by inducing oxidative stress and excessive production of reactive oxygen species (ROS) (Ferreira *et al*., 2020; Hong *et al*., 2020; Peng *et al*., 2020; Pratama *et al*., 2020). Elevated levels of ROS trigger signaling cascades such as mitogen-activated protein kinases (MAP kinases), promote auto-phosphorylation in the Epidermal Growth Factor (EGF) receptor, and affect the nuclear factor-κB (NF-κB). NF-κB activation stimulates the expression of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-a), interleukin-1 alpha (IL-1α), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), IL-8 and IL-6, leading to inflammation (Divya *et al*., 2015; Kuo *et al*., 2015; Shabunin *et al*., 2019).

Anti-melanogenesis effect of XG leaf cream

The impact of UVB radiation on melanogenesis can be observed through an increase in melanin content in the dorsal skin of guinea pigs (Idana *et al*., 2022). Histologically, XGL 15% showed no significant difference in melanin content compared to HQN, with a *p*-value of 0.828 (p > 0.005). The mean melanin content in XGL 15% (10.00±4.36) was lower than those in HQN (10.67±2.08), CBG (26.33±2.52), XGL 5% (19.33±5.51), and XGL 10% (19.00±2.65) (Figure 3) (Table III).

UV radiation induces direct DNA damage in keratinocytes. This DNA damage triggers the activation of P53 and subsequent expression of proopiomelanocortin (POMC). POMC acts as a precursor protein for α-MSH. The binding of α-MSH to MC1R initiates adenylyl cyclase activity, converting ATP to cyclic adenosine monophosphate (cAMP). This process elevates cAMP levels, thereby activating protein kinase A (PKA). PKA then activates microphthalmia-associated transcription factor (MITF) in melanocytes as a crucial transcription factor in melanogenesis.

Table III: Anti-inflammatory and anti-melanogenic effect of XG leaf-extract cream

Skin assessment	Experimental group		(x̄±SD)	*p*-value
	Positive control group (x̄±SD)	Comparison group		
Epidermal Thickness	24.25±1.62	Negative control group	39.91±1.84	0.001
		5% XGL-treated group	33.29±5.66	0.003
		10% XGL-treated group	27.72±1.12	0.165*
		15% XGL-treated group	14.23±0.95	0.001
Melanin	10.67±2.08	Negative control group	26.33±2.52	0.001
		5% XGL-treated group	19.33±5.51	0.016
		10% XGL-treated group	19.00±2.65	0.019
		15% XGL-treated group	10.00±4.36	0.828*

mean±standard deviation (SD), *not significant (p > 0.05) compared to the positive control group.

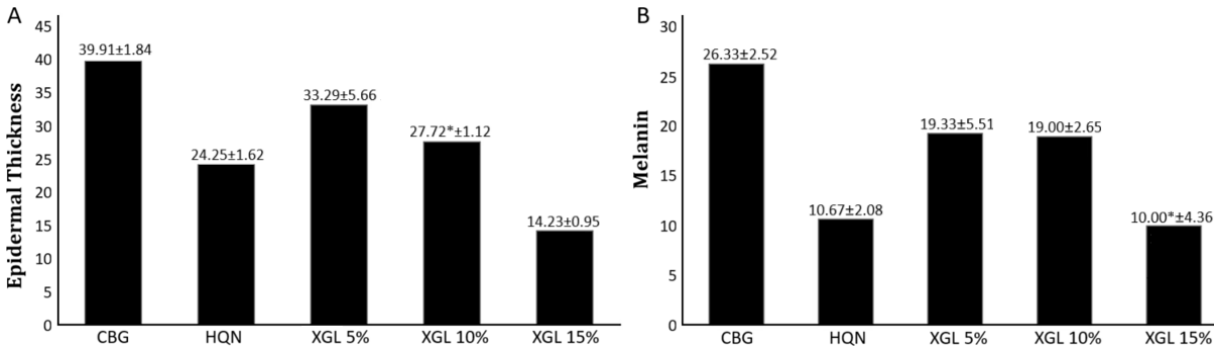

Figure 3. (A) The mean value of epidermal thickness at day 14 after radiation. (B) The mean value of melanin at day 14 after radiation. Abbreviations: CBG, Cream base group; HQN, Hydroquinone cream; XGL 5%, *X. granatum* leaf extract cream 5% concentration; XGL 10%, *X. granatum* leaf extract cream 10% concentration; XGL 15%, *X. granatum* leaf extract cream 15% concentration; (*) not significant (p>0.05) compared with positive control group.

MITF regulates the expression of structural proteins and melanogenic enzymes, including tyrosinase, tyrosinase-related protein-1 (TYRP-1), tyrosinase-related protein-2 (TYRP-2), and dopachrome tautomerase (DCT). These enzymes contribute to synthesizing eumelanin (brown to black pigment) and pheomelanin (yellow to red pigment). Melanin acts as the primary photoprotective agent in the skin against UV radiation. Melanin pigment is deposited in the epidermis to prevent DNA damage to keratinocytes and UV-induced carcinogenesis. The skin needs to be protected from excessive UV radiation to mitigate acute and chronic damage (Frank, 2021; Hida *et al*., 2020; Horrell *et al*., 2016; Nishi *et al*., 2020; Tagashira *et al*., 2015).

The cream formulated with *Xylocarpus granatum* leaf extract exhibits antioxidant, anti-inflammatory, and anti-melanogenic properties. The presence of flavonoids, tannins, and phenols in XGL extract contributes to its antioxidant potential by scavenging free radicals and inhibiting the processes of hyperkeratosis and melanogenesis induced by ROS (Feng *et al*., 2014). Moreover, the flavonoids, tannins, and saponins found in XGL extract demonstrate potential as tyrosinase inhibitors, an enzyme involved in the melanogenesis process (Gazali *et al*., 2014; Trisnawati *et al*., 2019).

Xylocarpus granatum leaves are a potential natural source of protecting the skin from the effects of UVB radiation with various active compounds. GC-MS chromatography analysis revealed the presence of 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (also known as methyl linolenate), as the most abundant compound in the ethanol leaf extract of *Xylocarpus granatum*. Methyl linoleate in the extract is crucial for having antioxidant, anti-inflammatory, and anti-melanogenic properties (Abdel-Hady *et al*., 2018; Ko *et al*., 2018; Tel *et al*., 2013; Tundis *et al*., 2019). Methyl linoleate, a common polyunsaturated fatty

acid (PUFA), exhibits anti-inflammatory effects by inhibiting the production of Nitric oxide (NO) and suppressing the expression of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and NOS2. NO production is associated with tissue damage in acute and chronic inflammations. Additionally, the anti-inflammatory activity of methyl linoleate involves downregulating the expression of the NF-kB subunit p50 (Saiki *et al*., 2017; C. C. Yang *et al*., 2017). Methyl linoleate demonstrates anti-melanogenic properties reducing melanin content by decreasing the expression of MITF, tyrosinase, and TRP1 proteins, and diminishing intracellular tyrosinase activity. This insight suggests that the anti-melanogenic effects of methyl linoleate can be attributed to the inhibition of MITF transcriptional activation (Ko *et al*., 2018; Tel *et al*., 2013).

The application of XG leaf extract topically demonstrated significant antioxidant, anti-inflammatory, and anti-melanogenic effects, as evidenced by the IC50 value, SPF value, and the variables of epidermal thickening and melanin contents. These responses can be attributed to various compounds detected in the gas chromatogram analysis of the XG leaf extract. However, further research is warranted to elucidate the underlying mechanisms of the anti-inflammatory and anti-melanogenic properties of the XG leaf extract. Additionally, evaluating the safety profile of the extract is crucial, considering that anti-inflammatory and anti-melanogenic agents often exhibit potential side effects that should be carefully considered.

CONCLUSION

Xylocarpus granatum leaf-extract cream, enriched with its bioactive constituents, exhibits protective properties against UVB-induced skin damage in male guinea pig models. The compound 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- was identified as the most abundant compound, possessing potent antioxidant, anti-inflammatory and anti-melanogenic activities. The XGL 15% cream was more effective in reducing epithelial thickness than HQN. Additionally, it was equally effective in reducing melanin contents compared to HQN. Furthermore, topical administration revealed a protective effect against UVB-induced skin inflammation. These findings provide a basis for further exploration of the potential role of Xylocarpus *granatum* as an adjunct therapeutic approach for managing sunburns.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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