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UV-Protection Effect of Nanoemulgel Formulation from *Moringa oleifera* Lam. Leaf Extracts in *Rattus novergicus*

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Abstract: Exposure to sunlight (UV radiation) can harm the skin, leading to cancer and tissue damage. The body's natural defense involves activating hormones that stimulate melanin synthesis to reduce cellular damage. *Moringa oleifera* Lam. leaves, rich in flavonoids and phenolic compounds, are promising UV-protection agents. This study assessed the efficacy of nanoemulgel formulations containing *M. oleifera* leaf extract through *in vitro* and *in vivo* assays. Twenty-five rats were divided into five groups, including formulations with 2%, 3%, and 4% *M. oleifera* extract, a negative control, and a positive control with Azarine Sunscreen Gel SPF 45. The nanoemulsions had appropriate size and quality, with the highest SPF observed at 4% concentration (7.95±0.06). According to FDA classification, these formulations fall within the 'extra' SPF category (6<SPF<8). However, melanin scores in the nanoemulgel groups were comparable to the untreated control, and the 4% concentration (44.4±16.3) did not match the protection provided by commercial sunscreen (10.0±1.58). These findings suggest *M. oleifera* has potential for photoprotection, but further optimization is needed.

Keywords: Histology; Melanin; *Moringa oleifera*; Nanomaterials; Photoprotection

Introduction

Indonesia's equatorial location results in consistent, year-round exposure to sunlight, leading to increased risks of skin damage due to ultraviolet (UV) radiation. Prolonged exposure to sunlight (UV rays) can have adverse effects on the skin, activating hormones that stimulate melanin pigment synthesis and resulting in darker skin (Brenner & Hearing, 2008; Maddodi et al., 2012; Yamaguchi & Hearing, 2006). Sun-damage skin often leads to mild tissue damage such as erythema, edema, sunburn, tanning, and hyperplasia (Balkrishna et al., 2023; Latcuba et al., 2022; Lubis et al., 2023; Setyowiranti & Darmaputra, 2023). Severe ultraviolet damage can cause photoaging and skin cancer (Irawan et al., 2022; Wijoyo & Wahyuniari, 2023). To date, solar ultraviolet (UV) radiation exposure is considered the primary cause of extrinsic skin aging (Siregar et al., 2024).

Sunscreens are cosmetic preparations used to reflect or actively absorb sunlight, particularly in the ultraviolet and infrared regions, thereby preventing skin disorders caused by UV rays (Saucedo et al., 2020). Recently, there has been a growing preference for natural ingredients in sunscreen formulations due to their perceived safety and lower risk of adverse effects compared to synthetic chemicals (Djunaidy & Damayanti, 2023; Suyono et al., 2023). Natural products like aloe vera, cucumber, and coconut pulp have demonstrated superior UV absorption and reduced skin irritations compared to their synthetic counterparts (Irimpan, 2020). Despite this, synthetic sunscreens continue to dominate the market due to their favorable benefit-risk profile and efficiency in mass production. Synthetic UV filters, such as benzophenone-3, have raised concerns due to their potential for genotoxicity, photoallergic reactions, and environmental impact, leading to a growing interest in natural alternatives like

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gadusol (Carvalho et al., 2023). Besides sun protection, natural sunscreens are valued for their healing, softening, and rejuvenating effects (Xie et al., 2024). However, both natural and synthetic sunscreens have their drawbacks: synthetic options can cause photoallergic dermatitis and contribute to environmental pollution, while natural alternatives face challenges in scaling up production. Natural ingredients are considered safer for use and have fewer negative impacts compared to chemical ingredients (Lister et al., 2022).

Moringa tree (*Moringa oleifera* Lam.) is a perennial tree species with high antioxidant activity, especially in its leaves. Traditionally, *M. oleifera* is widely used for its nutritional value and medicinal properties, including antibacterial, antioxidant, immunomodulatory, anticancer, antidiabetic, and photoprotective effects in various parts of the plant (Afriza et al., 2023; Handayani et al., 2022; Isnaini et al., 2023; Liu et al., 2022; Muharraran et al., 2023; Stohs & Hartman, 2015). The leaves of *M. oleifera* are rich in phenolic acids, flavonoids, glucosinolates, and isothiocyanates (Hassan et al., 2021; Maldini et al., 2014). Phenolic and flavonoid compounds are secondary metabolites produced in response to environmental conditions, and one of their functions is UV protection. Flavonoids in *Moringa* leaves absorb UV radiation and protect photosensitive compounds in the leaves (Jimtaisong, 2013). This study aims to develop a nanoemulgel formulation of *M. oleifera* leaf extract, exploring its potential as a topical treatment or sunscreen with melanin-inhibiting properties.

Method

Experimental Animals

The experimental animals used in this study were male Wistar rats (*Rattus norvegicus* L.), each weighing approximately 200 grams and aged between 2 to 3 months, all in healthy condition. The research process flow is visualized in a flowchart, as shown in Figure 1. The animals were maintained on a standard pellet diet using HPS 511, which contains (w/w): 20% protein, 5% fat, 45% starch, 5% crude fiber, and 4% ash. The rats were given drinking water *ad libitum*. The rats were housed in plastic containers with rice husk bedding and wire mesh covers, equipped with hanging food and water containers. The cages were placed in a naturally ventilated room, and the animals were acclimated for 14 days. Prior to the treatments, all male Wistar rats experienced a seven-day acclimatization period at the Animal House, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia. This acclimatization allowed the rats to adapt to their new environment, food, and water. This study were ethically supervised and approved by the Health Research Ethics Committee of Universitas Prima Indonesia, Medan, Indonesia, as documented in the Health Research Ethical Eligibility Statement No. 020/KEPK/UNPRI/1/2024.

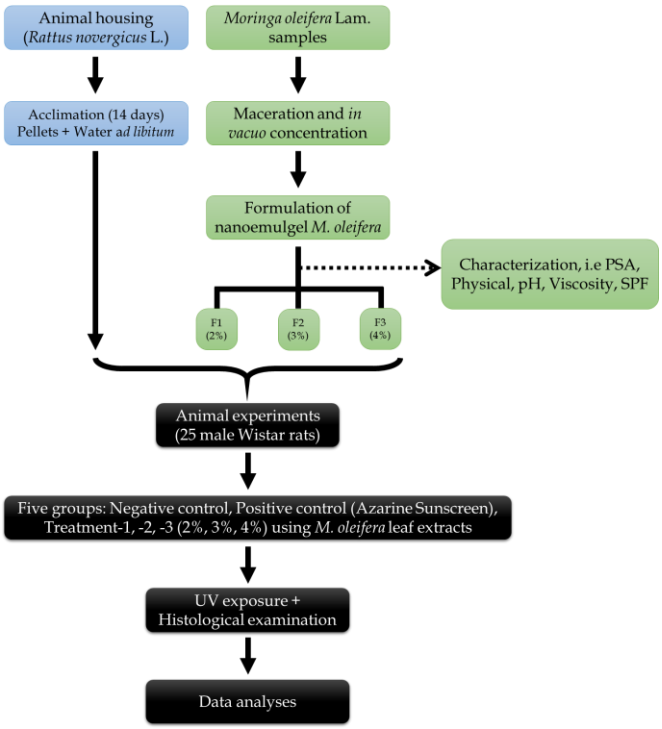

Figure 1. Flowchart of experimentation

Plant Collection and Extraction

Moringa oleifera or *kelor* leaves were collected from Keude Bayu Village, Syamtalira Bayu Subdistrict, North Aceh Regency. A total of 5 kg of *kelor* leaves were dried in an oven at 55°C. Once completely dried, the leaves were sorted and ground into a powder, yielding 1,416 g, which was then extracted using the maceration method. Ethanol was used as the solvent at a ratio of 1:3 (w/v). The maceration process was conducted with 14 L of 96% ethanol for 3×24 h with occasional stirring, followed by filtration. The residue was re-macerated with 7 L of 96% ethanol for 24 h. This extraction process was repeated twice with the same amount of solvent and duration to maximize the yield. The filtrate obtained was evaporated *in vacuo* at 40°C to remove the solvent. The yield of crude *kelor* leaf extract was 170 g, which was equivalent to 12.14% (w/w).

Formulation of Nanoemulgel from M. oleifera leaf Extract

The preparation of nanoemulgels involved three stages: the formulation of the nanoemulsion, the preparation of the gel base, and the incorporation of the nanoemulsion into the gel base. Three formulations of *kelor* leaf extract into nanoemulgel were prepared with compositions presented in Table 1.

Table 1. Composition of Nanoemulsion Formula of *M. oleifera* leaf Extracts

Materials	Formulation (%, w/w)		
	F1	F2	F3
M. oleifera O)	2	3	4
Nipagin® O)	0.18	0.18	0.18
Glycerine W)	10	10	10
Tween 80 W)	30	30	30
PEG W)	27	27	27
DW W) G)	100	100	100
Carbopol 940 G)	0.5	0.5	0.5
TEA G)	1	1	1

PEG: Polyethylene glycol, DW: Distilled water, TEA: Triethanolamine, O): Oil phase, W): Water phase, G): Gel base.

Moringa oleifera leaf extract was dissolved in 96% ethanol and mixed with liquid paraffin (oil phase). A mixture of Tween 80 and PEG 400 (water phase) was then added to the oil phase and homogenized using a homogenizer at 5000 rpm for 1 h (mixture). Methyl paraben or Nipagin® was dissolved in 96% ethanol and gradually added to the mixture while continuously stirring with the homogenizer at 5000 rpm for 1 h. This was followed by sonication at 40°C for 1 h. For the gel base preparation, carbopol 940 was used as the gelling agent and was dispersed in hot water at 80-100°C. The carbopol 940 was allowed to swell for 24 hours, then TEA was gradually added and ground until a clear gel base was formed. Then, nanoemulsion was incorporated into the gel base while homogenizing at 3000 rpm for 5 min. Once all the nanoemulsion was added, the speed was increased to 5000 rpm and stirred for another 5 min, followed by sonication at 40°C for 1 h. Nanoemulgels from each formulation with three replicates were then subjected to further analysis.

Characterization of M. oleifera Nanoemulgels

The particle size of the nanoemulgels containing *M. oleifera* leaf extract from each formulation was determined using Particle Size Analyzer (PSA, Analysette 22 NanoTec, Fritsch, Germany). Organoleptic properties and homogeneity of the nanoemulgels were assessed qualitatively. Acidity or pH value of the nanoemulgels was measured with a digital pH-meter. Viscosity was evaluated using a Brookfield Viscometer. Dispersive power of the nanoemulgels was physically measured over a duration of 60 sec on a flat surface.

Determination of Sun Protection Factor (SPF) of Nanoemulgels

The SPF value was determined by measuring the absorbance of the solution for each formulation at every 5 nm interval using a UV-Vis spectrophotometer within the wavelength range of 290-320 nm. A 5 g sample of the preparation was dissolved in 50 ml of 70% ethanol. Three replicates were performed for each formulation. The absorbance results were recorded and calculated using the following formula (Mansur et al., 1986):

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

Where: *CF* = Correction factor (10), *EE* =Erythemogenic effect of radiation within wavelength, *I* = Solar intensity spectrum, *Abs* (λ) = Spectrophotometric absorbance value at certain wavelength. The value of *EE* and *I* are constants based on (Sayre et al., 1978).

Experimental Design and Procedure

After the acclimatization period and consumption of standard pellet feed, 25 male Wistar rats) were randomly divided into five groups, each consisting of 5 rats, and subjected to treatments for 28 days as follows: Negative Control group was exposed to ultraviolet-B (UV-B) radiation for 28 days without any treatment, Positive Control group was exposed to UV-B and applied with Azarine Sunscreen gel daily for 28 days, Treatment-1 group was exposed to UV-B and applied with F1 nanoemulgel daily for 28 days, Treatment-2 group was exposed to UV-B and applied with F2 nanoemulgel daily for 28 days, and Treatment-3 group was exposed to UV-B and applied with F3 nanoemulgel daily for 28 days. The dorsal hair of all rats was shaved using electric and manual razors over a 2×2 cm² area. The nanoemulgel was applied evenly at a dosage of 2 mg/cm² to the designated area. After 20 min for absorption, UV-B exposure (using Exoterra UVB 200 13 watt lamp) was administered for approximately 6 h at a distance of 30 cm to induce erythema, with a following reapplication of the topical agent 4 h later to allow for reactive oxygen species (ROS) formation. This procedure was repeated daily for 28 days. After the 28-day period, the rats were euthanized with an overdose of ketamine (125 mg/kg BW) intramuscularly in an anaerobic jar 48 h after the last UV exposure. Skin samples were collected from the dorsal area, cleaned of hair, and cut to a thickness of approximately 2 mm down to the subcutaneous layer, measuring 2×2 cm. Histopathological slides were prepared, and melanin pigments were counted in ten microscopic fields at 100× magnification.

Histological Examination

Histopathological preparations were made through following steps: Skin samples were fixed in 10% formalin, dehydrated, and sequentially cleared using a series of solutions (thrice in 10% formalin, 70% alcohol, 96% alcohol, thrice in absolute alcohol, thrice in xylol, and twice in liquid paraffin) over a period of 23 h. The samples were then embedded in liquid paraffin, cooled for 30 min, and sectioned using a microtome. Before

mounting, the sections were stained using the Hematoxylin-Eosin (HE) method. The sections were immersed in xylol-I,-II, and -III for 5 min each, followed by immersion in absolute alcohol-I and -II for 5 min each. Prior to HE staining, the sections were soaked in distilled water for 1 min. The samples were then soaked again in distilled water for 1 min, followed by 5-7 min in 10% acid alcohol, and rinsed twice in distilled water for 15 min. Afterward, the sections were stained with eosin. The stained sections were then immersed in 96% alcohol four times for 3 min each, and finally cleared in xylol-I and -II for 5 min each.

Data Analysis

The numerical data from each treatment and replication were presented as mean ± standard deviation. Differences in means between groups were analyzed using one-way ANOVA, followed by post-hoc tests for multiple comparisons using Tukey's Honestly Significant Difference (HSD) test if significant ($P<0.05$).

Result and Discussion

Particle size of M. oleifera leaf Extract Nanoemulsions

The process of creating nanoemulsions and nanoemulgels started with the drying of the simplicia material, followed by extraction, *in vacuo* concentration, nanoemulsion formulation, and finally the preparation of the gel formulation. The particle sizes for each nanoemulsion, measured using a Particle Size Analyzer (PSA), were 73.15 nm, 87.21 nm, 112.72 nm, and 143.05 nm for the control, 2%, 3%, and 4% *M. oleifera* leaf extract formulations, respectively (Figure 2). The definition of nanomaterials differs among researchers; however, it typically encompasses materials with at least one dimension smaller than 100 nm (Pelleg, 2021). Furthermore, Shah et al. (2010) described that nanoemulsion droplet sizes typically range between 20 and 200 nm, exhibiting narrow size distributions.

Based on theoretical considerations, only the control and the 2% formulation of *M. oleifera* leaf extract that are categorized as nanoemulsions, while the 3% and 4% formulations either fall under the category of microemulsions or nanoemulsions. Nevertheless, the study will continue to assess their efficacy as topical agents. Formulation of nano- and microemulsions from *M. oleifera* and its relative has been reported in several studies using various materials. Mattar et al. (2024) developed stable nanoemulsions using sodium caseinate and *Moringa* seed oil, with particle sizes ranging from 125.80 to 167.50 nm, for functional ice cream production. Jusnita and Nasution (2019) created nanoemulsions from *Moringa* leaf extract using Tween 80 as a surfactant, achieving particle sizes as small as 7.9 nm. Das et al. (2020) formulated a tri-herbal nanoemulsion including *M. oleifera* leaf extract, with a particle size of 28.72 nm and good stability. Lourith et al. (2016) also developed stable microemulsions using *M. oleifera* oil, with particle sizes between 83.75 and 286.76 nm. The differences in techniques and types of materials dominantly influenced the quality and size of the nanomaterials produced. This also accounts for the variations in the particle sizes of the nanoemulsions obtained in this study.

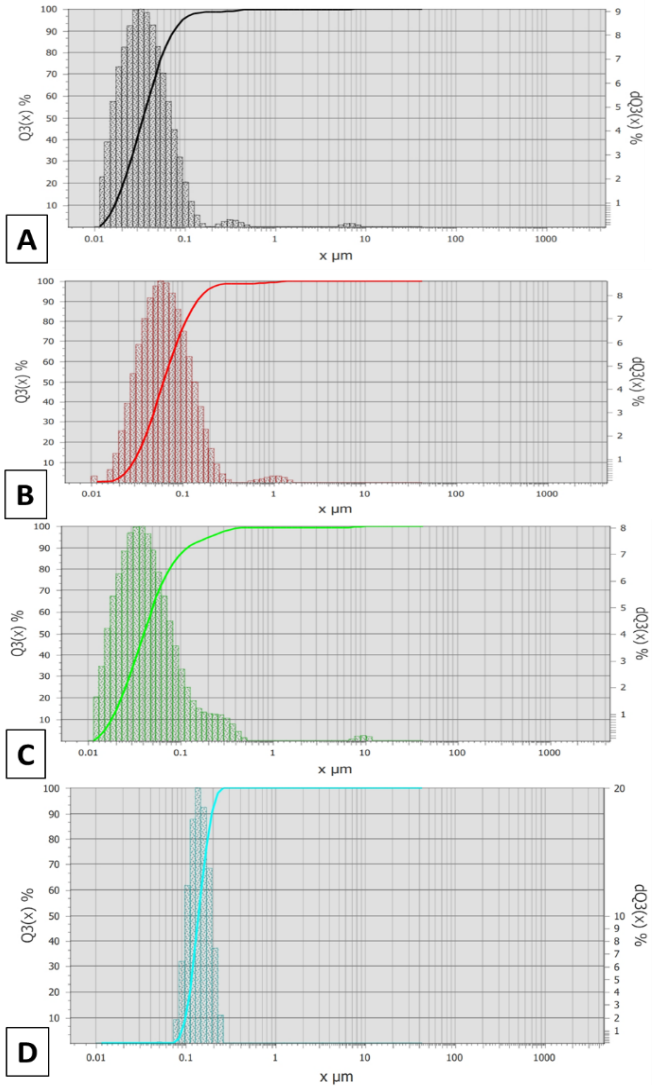

Figure 2. Particle size distribution for nanoemulsions composed with *Moringa oleifera* leaf extract at (A) 0%/ Control, (B) 2%, (C) 3%, and (D) 4% concentrations

Physical Attributes of M. oleifera leaf Extract Nanoemulgels

The physical quality of each nanoemulgel formulation was examined per parameter. A summary of the nanoemulgel preparation steps is illustrated in Figure 3. Organoleptically, there were no differences in color among the nanoemulgel preparations with 2%, 3%, and 4% concentrations; all displayed a dark green hue due to the *M. oleifera* leaf extract. Each concentration of

the extract imparted a distinctive aroma. The nanoemulgels exhibited a smooth texture, easy spreadability, a semi-solid or gel-like consistency, and a non-sticky feel. Visually, the nanoemulgels appeared homogeneous, indicating that the ingredients used in the gel preparation were well-mixed. The acidity levels (pH) and dispersive power (spreadability) of each formulation are shown in Table 2.

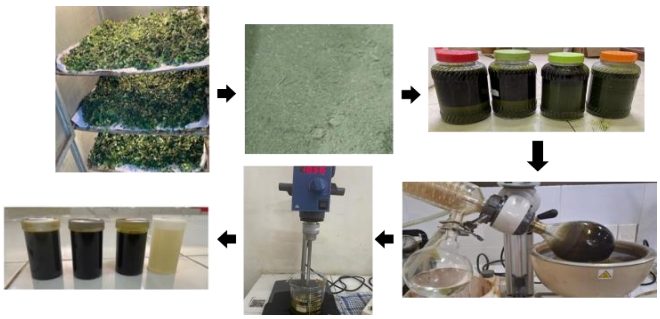

Figure 3. Documentation of *M. oleifera* leaf extract preparation sequence with the final product consisted of four nanoemulgels

Table 2. pH evaluation, dispersive power, and viscosity of nanoemulgels from *M. oleifera* leaf extracts

Nanoemulgels	Parameter (mean±S.D)		
	pH	Dispersion (cm)	Viscosity (cP)
Control	5.82±0.06[a]	5.53±0.15[b]	930±5.10[a]
2% *M. oleifera*	5.51±0.07[b]	6.80±0.20[a]	773±9.58[b]
3% *M. oleifera*	5.22±0.06[c]	5.90±0.36[b]	820±2.70[c]
4% *M. oleifera*	5.01±0.08[d]	5.43±0.31[b]	856±897[d]

Different letters in the same column denotes statistical significance ($P \leq 0.05$) based on Tukey's HSD post-hoc test.

The pH values of each nanoemulgel varied significantly ($F_{3,8}$ = 79.82, P = 0.000) with the highest value observed in the control nanoemulgel and the lowest in the 4% formulation. Spreadability also differed significantly among the formulations ($F_{3,8}$ = 16.20, P = 0.001), with the widest spread observed in the 2% formulation and the narrowest in the 4% formulation. The highest viscosity was found in the control nanoemulgel, while the lowest viscosity was in the 2% formulation. The differences in mean values for each formulation were also statistically significant ($F_{3,8}$ = 254.17, P = 0.000).

According to SNI 16-4399-1996, the ideal pH for gel formulations should match the skin's pH, which ranges from 4.5 to 8.0. A gel with a pH outside this range can cause skin irritation. Other studies suggest that the optimal pH for topical formulations applied to human skin should be slightly acidic, typically ranging from 4 to 6 (Lukić et al., 2021). The natural pH of the stratum corneum is between 4.1 and 5.8, which is crucial for maintaining skin barrier function (Proksch, 2018). Therefore, it is essential for topical lotions or gels to fall within this pH range. Dispersive power and viscosity of a product are tested to quantify the resistance of a fluid to flow; higher viscosity indicates greater resistance. Increased viscosity prolongs retention time at the application site but reduces spreadability. These findings suggest that higher concentrations of *M. oleifera* leaf extract result in larger spreading areas due to increased viscosity, thereby enhancing spreadability upon application. Binder et al. (2019) stated that a gel with high viscosity and low spreadability may consequently result in reduced skin penetration.

SPF of M. oleifera leaf Extract Nanoemulgels

The UV-protecting effects of nanoemulgels formulated from various concentrations of *M. oleifera* leaf extract were evaluated based on their Sun Protection Factor (SPF). Absorbance in the wavelength range of 290-320 nm was measured and calculated using predefined constants and correction factors (Figure 4A). SPF values varied significantly among formulations ($F_{2,6}$ = 215.55, P = 0.000), with the highest observed at 4% concentration (7.95±0.06), followed by 3% (7.69±0.02), and 2% (7.25±0.02). This indicates a concentration-dependent SPF property (Figure 4B). According to the classification by the Food and Drug Administration (FDA), the SPF properties of nanoemulgels from this study fall within the 'extra' category (6<SPF<8).

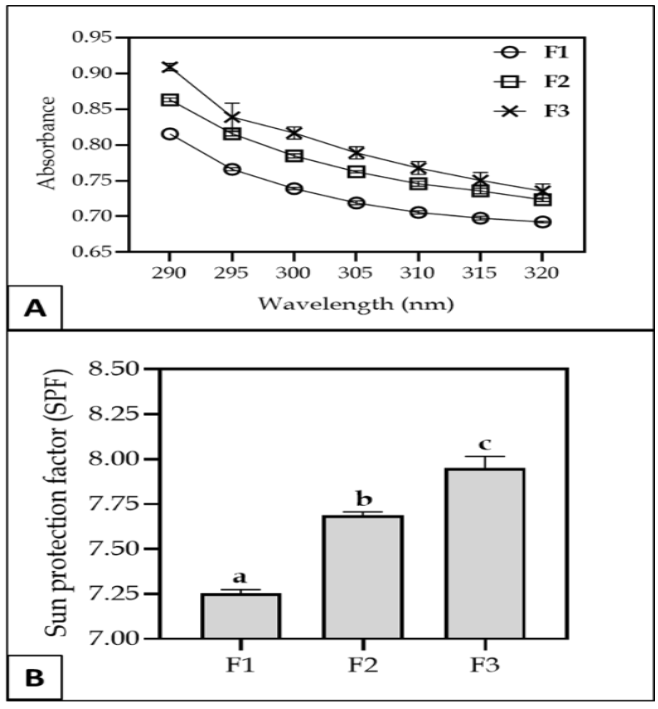

Figure 4. (A) Absorbance reading within UV wavelength (290-320 nm), (B) Sun protection factor (SPF) of nanoemulgel from *M. oleifera* leaf extracts at different concentrations: F1 (2%), F2 (3%), F3 (4%). Different letters denote statistical significance at $P \leq 0.05$ from Tukey's HSD post-hoc test

Previous studies have reported SPF values for *Moringa*-based formulations: Gaikwad and Kale (2011) reported SPF 2.04 for *Moringa* oil cream, Santos et al. (2021) obtained SPF values ranging from 5.1 to 7.6 for *M. oleifera* oil dichloromethane fraction using a 0.5% concentration. Baldisserotto et al. (2018) achieved SPF 2 (providing 50% UV-B protection) with *Moringa* leaf extract concentrations of 2-4%. The SPF capacity observed may be attributed to the phenolic content and antioxidant properties derived from bioactive metabolites present in *M. oleifera* (Hassan et al., 2021; Pakade et al., 2013). Gimenis et al. (2018) found that fresh leaf and flower extracts of *M. oleifera* exhibited significant antioxidant and photoprotective activities due to their phenolic and flavonoid content. Varsha et al. (2018) reported strong UV absorption by ethanolic leaf extracts, indicating their potential as anti-solar agents.

Histopathology and Melanin Count of UV-Exposed Rat Skins

Histological examination of UV-exposed skin before and after treatment was conducted to observe changes in the skin tissue of rats, as shown in Figure 5.

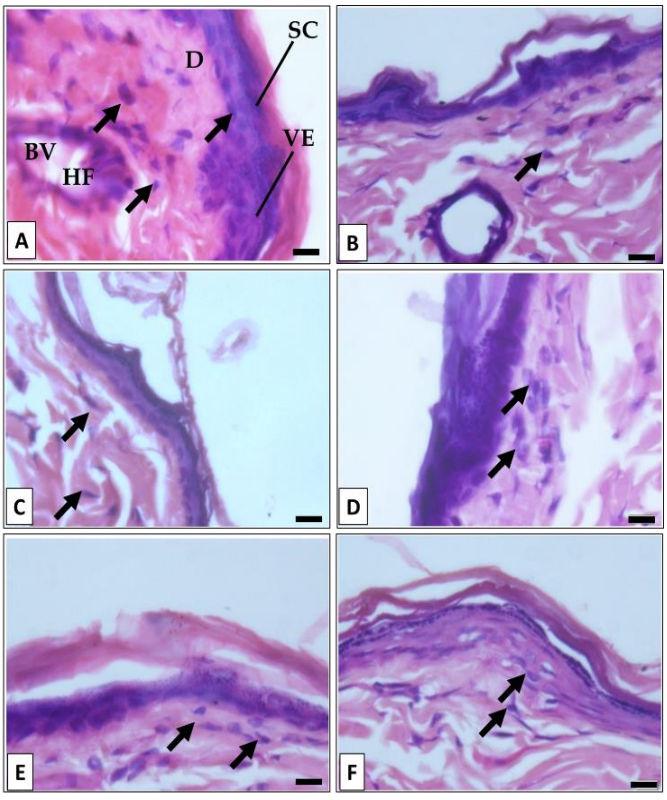

Figure 5. Histology of (A) UV-exposed rat skin without treatment, (B) normal rat skin without sunscreen, (C) Azarine Sunscreen gel treatment, (D), (E), (F) nanoemulgel treatment of *M. oleifera* leaf extract at 2%, 3%, and 4%, respectively. Note: BV = blood vessel, D = dermis, HF = hair follicle, SC = stratum corneum, VE = viable epidermis, arrows showing melanin deposits

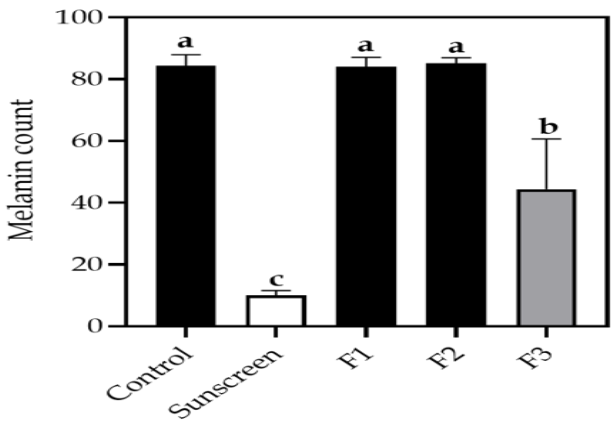

Figure 6. Melanin score in UV-exposed rat skin under control, commercial sunscreen, and nanoemulgels formulated of *M. oleifera* leaf extracts at concentration of 2% (F1), 3% (F2), and 4% (F3)

Intense UV-B exposure triggers melanin synthesis and cell deposition towards the epidermis to block UV penetration and prevent damage to deeper tissues, as seen in Figure 5A. The application of commercial sunscreen and nanoemulgels on UV-exposed skin visually showed a reduction in melanin. Melanin quantification and scoring were then performed to assess the effectiveness of the nanoemulgels compared to the control (Figure 6). The number of melanins varied significantly depending on the treatment administered ($F_{4,20}$ = 97.01, P = 0.000). Melanin score in the control group (84.4±3.58), which was exposed to UV without any treatment, was not significantly different from the nanoemulgel treatments F1 (84.0±3.08) and F2 (85.2±1.79), formulated with 2% and 3% *M. oleifera* leaf extract, respectively. The nanoemulgel treatment F3, with the highest *M. oleifera* concentration at 4% (44.4±16.3), still did not match the protective effect provided by the commercial sunscreen (10.0±1.58).

The significant difference in results is attributed to the higher SPF and superior protection of commercial sunscreen, which contains pure active ingredients, while the crude extract of *M. oleifera* may include compounds that are less synergistic in photoprotection. Other studies have explored natural ingredients as sunscreen agents. Harahap et al. (2022) reported a decrease in the number of melanocytes (melanin) of guinea pig skin after treatment with strawberry extract. Tobing et al. (2020) reported that black cumin (*Nigella sativa* L.), at a topical concentration of 0.75%, reduced melanin in rat skin tissue. Amelia et al. (2021) also found that red spinach extract ointment (*Amaranthus tricolor* L.), at a concentration of 10%, improved skin pigmentation levels before and after treatment. Future research could focus on UV-induced skin fibroblast cells to observe physiological and genetic responses related to

inflammation and aging (Girsang et al., 2021). In addition, efforts should be directed toward improving the quality of nanoemulgel through formulation optimization and the extraction of other active *Moringa* components.

Conclusion

In conclusion, while *Moringa oleifera* Lam. leaves, known for their high flavonoid and phenolic content, demonstrate potential as natural UV-protection agents, the current nanoemulgel formulations require further optimization. The study revealed that the 4% *M. oleifera* extract formulation achieved the highest SPF value within the 'extra' protection category, yet its melanin inhibition efficacy was still inferior to that of the commercial sunscreen. These results indicate that although *M. oleifera* shows promise for photoprotection, further study and formulation adjustments are necessary to enhance its effectiveness as a topical agent for skin protection against UV radiation.

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Author Contributions
Conceptualization and design of research work (LC, CNG); Implementation of field/laboratory experiments and data collection (ATA); Data analysis and interpretation (ATA, LC); Manuscript preparation (ATA).

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Conflicts of Interest
The authors declare no conflict of interest.

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