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Research Article

Antibacterial, Antifungal, Antioxidant, and Photoprotective Analysis of Mangrove Extracts as Additives Ingredients in a Cosmetic Cream

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ABSTRACT

Mangroves are a rich source of natural antioxidant compounds that can inhibit and reduce tissue damage caused by free radical activity. Currently, cosmetic creams are widely recommended for maintaining skin health and aesthetics, especially in protecting against the harmful effects of free radicals. This study applied mangrove extract as an additive ingredient in a cosmetic cream, serving as an antibacterial, antifungal, antioxidant, and photoprotective agent. Mangrove samples were collected from the mangrove ecosystem in Tapak Village, Semarang, Indonesia. Three mangrove species were evaluated for their bioactivity, and the species with the highest bioactivity was selected for use in the cream formulation. Antibacterial and antifungal activities were tested using the disc diffusion method, antioxidant activity was assessed using the DPPH method, and photoprotective activity was determined using UV spectrophotometry, with analysis based on the Mansur mathematical equation. The cream was evaluated for its characteristics, including spreadability, homogeneity, sensory properties, pH, sun protection factor (SPF), phytochemical content, stability, and microbial contamination. An *in vivo* was conducted to assess the cream's effectiveness on white mice. Results indicated that *Avicennia marina* leaf extract exhibited the highest bioactivity compared to the other two species, *Rhizophora mucronata* and *Bruguiera gymnorrhiza*. Consequently, *A. marina* leaf extract was selected as the active additive ingredient in the cream formulation. Characterization tests demonstrated that the cream was stable and met standard criteria for quality. The *in vivo* analysis revealed that the mangrove leaf extract cream significantly prevented epidermal thinning, reduced neutrophil counts, and preserved fibroblast numbers.

1. Introduction

Healthy skin is essential for most people today. However, many daily activities expose individuals to environmental factors that can harm the skin, such as cigarette smoke, air pollution, and ultraviolet (UV) radiation. Prolonged UV exposure poses significant risks to human skin, contributing to reduced skin elasticity, erythema, pigmentation, and even skin cancer. When skin cells are exposed to pollutants, skin characteristics can change, including pH alterations, sebum secretion rate, inflammation markers, collagen levels, and elastin content (Rembiesa *et al.* 2018; Chauhan & Gupta 2020).

By 2016, industrial production from natural raw materials in Indonesia increased by more than 30% (Aziliya 2016). The market for natural products, including cosmetics derived from natural raw materials, is in high demand due to their perceived safety: minimal synthetic chemical compounds and reduced environmental impact. Plant-based cosmetics continue to be researched and developed, as existing studies suggest this field holds great promise for the future (Hoang *et al.* 2021; Murargo 2021; Mahendra *et al.* 2022).

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Indonesia is home to some of the richest marine biodiversity in the world, including extensive mangrove ecosystems. With a coastline of 95,181 km, Indonesia possesses the largest mangrove area globally, covering 3.3 million hectares (Wagey *et al.* 2020; Arifanti *et al.* 2022). Mangroves typically thrive in muddy coastal wetlands, although some can also be found in lagoon areas and river estuaries that are inundated at high tide and free of inundation at low tide.

Complex abiotic factors (UV light, salinity, pH, and nutrient availability) and biotic factors (inter-species and intra-species relationships) play a crucial role in the mangrove ecosystem. As a result of these factors, mangroves have to adapt physically and produce secondary metabolites (Yeshi *et al.* 2022). These secondary metabolites are produced in response to extreme habitats and have been found to possess antimicrobial, antimalarial, anticancer, and antioxidant properties (Dewanto *et al.* 2018; Belhadj-Salah *et al.* 2022). Given these bioactive properties, mangrove leaves can be a promising source of ingredients for the cosmetics industry. Therefore, exploratory research on mangrove leaf extracts is warranted to assess their potential as antibacterial, antifungal, antioxidant, and photoprotective agents in developing cosmetic creams.

2. Materials and Methods

2.1. Sample Collection Site

Samples were collected from the mangrove ecosystem of Tapak Village, Tugu District, Semarang City, Indonesia. Astronomically, it is located at 110º17'15"E-110º22'4"E and 6º56'13"S-6º59'14"S. The leaves used as samples were still attached to the branches and intact, dark green in color, and neither yellow nor too old (Iranawati *et al.* 2020). Samples were stored in dark-colored ziplock bags and transported to the laboratory in a cool box maintained at ±4ºC (Nurjanah *et al.* 2020).

2.2. Sample Preparation

The samples were washed with running water and then rinsed with distilled water to remove residual dirt (Audah *et al.* 2018). The samples were air-dried for ten days at room temperature without exposure to direct sunlight. After that, all samples were ground into powder using a blender. The extraction method was based on Iranawati *et al.* (2020) and had some modifications. The samples were extracted by maceration using methanol solvents. Maceration was carried out for 3 × 24 h with occasional stirring; on the second and third days, the solvent was filtered, and the leaf powder was macerated again using a new solvent. The filtrate was then evaporated at 44ºC to obtain bioactive compounds.

2.3. Antibacterial and Antifungal Activity Test

The disc diffusion method was conducted according to Audah *et al.* (2018) with some modifications. The skin pathogenic bacteria used were *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while the fungi used were *Candida albicans* and *Malassezia furfur*. Bacteria cultures in Nutrient Broth and fungi cultures in Saboraud Dextrose Broth were adjusted to 1×10^8 CFU/ml concentration or equivalent to 0.5 McFarland turbidity standard. Pathogenic bacteria were spread using sterile cotton swabs on Mueller Hinton Agar. Pathogenic fungi were poured and spread with a sterile glass spreader on Saboraud Dextrose Agar. The paper discs were dripped with 50 µL extract with a 200 µg/ml concentration and placed on the agar media. The inhibition zone around the discs was measured using a caliper.

2.4. Antioxidant Activity Test

The test began with preparing a 100 µg/ml extract solution and 0.5 mM DPPH. The extract solution was combined with 1 ml of DPPH and then incubated in a dark room for 30 min. The absorbance value was measured using a UV-Vis spectrophotometer at a wavelength of 517 nm (Iranawati *et al.* 2020).

2.5. Photoprotective Activity Test

The photoprotective activity test was based on the method described by Dutra *et al.* (2004) with some modifications. The test measured the universal indicator Sun Protection Factor (SPF). The sample absorbance was measured using a UV-Vis spectrophotometer at 290-320 nm wavelength with a 5 nm interval. The SPF value was calculated using the Mansur mathematical equation.

2.6. Cream Preparation

Cream preparation followed the research of Mishra *et al.* (2014) with modifications. Mixing oil-soluble ingredients was carried out at a temperature of ±75°C, and at the same time, water-soluble ingredients were mixed at a temperature of ±75°C. After both preparations were homogeneous and reached the same temperature of ±70°C, the two preparations were mixed into a homogeneous plain cream. When plain cream reached a temperature of 40°C, additional ingredients were added slowly for ±15 min until the cream became homogeneous. The cream was made using leaf extract from a mangrove species that had

been tested for its antibacterial, antifungal, antioxidant, and photoprotective potential. The cream was made in four different concentrations of extract: 0% (F0), 2.5% (F1), 5% (F2), and 7.5% (F3).

2.7. Evaluation of Cream Characteristics

2.7.1. Spreadability Test

A 0.5 g cream was weighed and placed on a round glass, then another round glass was placed on top and left for 1 min. Then, the load of 50 g and 100 g were added, with a difference in the load's time every 1 min. The cream spread diameter was calculated and averaged (Sapiun *et al.* 2022).

2.7.2. Homogeneity Test

The homogeneity test was performed by weighing 0.1 g of cream, placing it on an object glass, and covering it with a cover glass. The cream was homogeneous if no particles or coarse grains appeared on the slide. The test was carried out three times (Sapiun *et al.* 2022).

2.7.3. Sensory Characteristics Test

The characteristics of the cream were assessed using the organoleptic method to evaluate the physical appearance, including color, aroma, and texture. It was carried out on plain cream and cream with added extract (Sapiun *et al.* 2022).

2.7.4. pH Test

A 0.5 g cream was weighed and dissolved in 50 ml distilled water. This test was carried out three times (Mishra *et al.* 2014).

2.7.5. Determination of SPF Value

The sample absorbance was measured using a UV-Vis spectrophotometer at 290-320 nm wavelength with a 5 nm interval. The SPF value was calculated using the Mansur mathematical equation (Dutra *et al.* 2004).

2.8. Determination of Formula for Advanced Characteristic Evaluation

For advanced evaluation, one formula from four types was selected first. The selection was based on the fulfillment of requirements (spreadability, homogeneity, and pH) and the advantages of each formula (based on SPF and sensory characteristics).

2.9. Advanced Evaluation

2.9.1. Phytochemical Screening

Phytochemical screening was conducted by observing the color and precipitation reactions in the sample after being treated with a reagent. Phytochemical screening included the identification of alkaloids, tannins, and saponins. The alkaloid test was performed using Dragendorff, Mayer, and Wagner reagents. The tannin test was performed using $FeCl_3$ reagents. The saponin test was performed by vigorous shaking and observing foam in the sample (Evania & Rakainsa 2023).

2.9.2. Centrifugal Test

A 10 g cream was weighed and placed in a centrifugation tube, which was rotated at 3,800 rpm for 5 h. The pH and microbial contaminant values before and after the centrifugal test were compared (Nurjanah *et al.* 2020).

2.9.3. Microbial Contamination Test

The microbial contamination test was performed using the total plate count method based on SNI 19-2897-1992. A 10 g cream was weighed sterilely into a glass jar, 90 ml of Buffered Peptone Water was added and then homogenized to obtain a 10^{-1} dilution. After that, dilutions 10^{-2} and 10^{-3} were prepared. From each dilution, 1 ml was pipetted and put into a petri dish, and then, approximately 12-15 ml of pre-cooled Plate Count Agar (PCA) was poured into each petri dish that already contained the sample and swirled to mix well. After the media solidified, the dish was incubated at 35ºC for 24 h in an inverted position. The dilution value was recorded, and all growing microbial colonies were counted.

2.9.4. Histological Analysis

The histological analysis referred to Hadiningrat *et al.* (2023), with modifications to the active ingredients. The animals used were 15 male, white rats *R. norvegicus* Wistar strain, aged two months. The animals were randomly divided into three groups (n = 5 per group). The three groups were control (without cream), plain cream (without mangrove extract), and cream with added mangrove leaf extract. All three groups of animals were exposed to UV-B light, but the control group was only exposed to UV-B light and did not receive any cream.

Plain cream and mangrove leaf extract cream were applied twice daily for 4 weeks, 20 min before and 4 h after irradiation. Cream application was continued on days without irradiation. Mice were euthanized 24 h after the last topical cream application. Back skin tissue was incised and hematoxylin-eosin (HE) stained to analyze the mean epidermal thickness, neutrophil

accumulation, and fibroblast expression. Diponegoro University Faculty of Medicine's Research Ethical Committee has approved every procedure in this *in vivo* study (No. 033/EC-H/KEPK/FK-UNDIP/IV/2024).

3. Results

3.1. Maceration Results

From 30 g of dried leaves extracted using 300 ml of methanol for 3 × 24 h, the highest yield was obtained from *A. marina* leaves (11.00%), followed by *R. mucronata* (7.67%) and *B. gymnorrhiza* (7.00%).

3.2. Antibacterial Activity

The largest inhibition zone against the growth of *P. aeruginosa* was derived from *A. marina* leaf extract (17.68±0.25 mm), followed by *B. gymnorrhiza* (14.68±0.38 mm) and *R. mucronata* (14.20±0.70 mm). The largest inhibition zone against *S. aureus* was derived from *A. marina* leaf extract (17.33±0.10 mm), followed by *B. gymnorrhiza* (14.62±0.52 mm) and *R. mucronata* (14.47±0.30 mm) (Table 1).

3.3. Antifungal Activity

The largest inhibition zone against the growth of *C. albicans* was derived from *A. marina* leaf extract (15.65±1.22 mm), followed by *R. mucronata* (15.45±0.95 mm) and *B. gymnorrhiza* (14.47±0.87 mm). The largest inhibition zone against *M. furfur* was derived from *R. mucronata* leaf extract (13.65±0.78 mm), followed by *A. marina* (13.38±0.88 mm) and *B. gymnorrhiza* (13.27±0.30 mm) (Table 2).

3.4. Antioxidant Activity

Based on the inhibition calculation in Table 3, *A. marina* extract had the highest free radical inhibition ability (50.15%). In contrast, *B. gymnorrhiza* had the lowest (37.82%). The Least Significant Difference (LSD) test showed that there was a significant difference between the mangrove leaf extracts of *A. marina* with *R. mucronata* and *A. marina* with *B. gymnorrhiza* ($p<0.05$).

3.5. Photoprotective Activity

The SPF test determined the effectiveness of mangrove leaf extract solutions of *R. mucronata, A. marina*, and *B. gymnorrhiza* with a concentration of 100 µg/ml against ultraviolet radiation. The absorbance and SPF values of mangrove leaf extracts are presented in Table 4. It was observed that the SPF value of *A. marina* extract was the highest (3.348), whereas the SPF value of *B. gymnorrhiza* was the lowest (2.219). The LSD test indicated that there was a significant difference in *A. marina* leaf extracts compared to the others ($p<0.05$).

Table 1. The inhibition zone of mangrove leaf extract against *P. aeruginosa* and *S. aureus*

Pathogenic bacteria	Zone of inhibition (ZOI) (mm)		
	R. mucronata	*A. marina*	*B. gymnorrhiza*
P. aeruginosa	8.20±0.70	11.68±0.25	8.68±0.38
S. aureus	8.47±0.30	11.33±0.10	8.62±0.52

Table 2. The inhibition zone of mangrove leaf extract against *C. albicans* and *M. furfur*

Pathogenic fungi	Zone of inhibition (ZOI) (mm)		
	R. mucronata	*A. marina*	*B. gymnorrhiza*
C. albicans	9.45±0.95	9.65±1.22	8.47±0.87
M. furfur	7.65±0.78	7.38±0.88	7.27±0.30

Table 3. Percentage inhibition of mangrove leaf extract

Mangrove species	Concentration (µg/ml)	Mean absorbance	Percentage inhibition (%)
R. mucronata	100	0.84±0.02[a]	43.20
A. marina	100	0.74±0.01[b]	50.15
B. gymnorrhiza	100	0.92±0.01[c]	37.82
Vitamin C	10	0.46±0.02	68.77

Different superscript letters indicate significant differences at the 95% confidence level

Table 4. SPF value of mangrove leaf extract with a concentration of 100 µg/ml

Mangrove species	Wavelength (λ)	Absorbance value	SPF
R. mucronata	290	0.297±0.001	2.906[a]
	295	0.291±0.001	
	300	0.289±0.001	
	305	0.290±0.000	
	310	0.293±0.001	
	315	0.294±0.000	
	320	0.287±0.000	
A. marina	290	0.325±0.006	3.278[b]
	295	0.326±0.005	
	300	0.325±0.006	
	305	0.328±0.006	
	310	0.331±0.006	
	315	0.332±0.005	
	320	0.329±0.006	
B. gymnorrhiza	290	0.217±0.005	2.156[c]
	295	0.213±0.005	
	300	0.212±0.005	
	305	0.215±0.005	
	310	0.219±0.005	
	315	0.223±0.004	
	320	0.224±0.005	

Different superscript letters indicate significant differences at the 95% confidence level

3.7. Evaluation of Cream Characteristics

For cream preparation, one leaf extract was selected first from three based on its potential as an antibacterial, antifungal, antioxidant, and photoprotective agent. Based on the results, *A. marina* leaf extract had more potential than the others.

3.7.1. Spreadability

The spreadability test was carried out to determine the spread of cream when applied to the skin. The spreadability of the cream was 4-5 cm (Table 5).

3.7.2. Homogeneity

The cream was semi-solid, light green to dark green, and homogeneous. All formulations meet the homogeneity. Homogeneous cream was characterized by the absence of coarse particles on the slide surface (Figure 1).

3.7.3. Sensory Characteristics

The sensory characteristics test was conducted to visually determine the color, aroma, and texture of the mangrove leaf extract cream. The results are in Table 6.

Table 5. Spreadability value of mangrove leaf extract cream

Formula	Load (g)	Diameter (cm)
F0	50	4.06±0.02
	100	4.18±0.02
F1 (2.5%)	50	4.38±0.03
	100	4.53±0.08
F2 (5%)	50	4.45±0.02
	100	4.50±0.03
F3 (7.5%)	50	4.50±0.03
	100	4.56±0.03

3.7.4. pH and SPF

Table 7 shows that the approximate pH value of 2.5% mangrove leaf extract cream was 7.30, 5% mangrove leaf extract cream was 7.14, and 7.5% mangrove leaf extract cream was 7.03. The cream was neutral, with a pH value of around 7. Table 7 also shows that F0 had the lowest SPF value (0.673), while F3 had the highest (6.983).

3.8. Determination of Formula for Advanced Evaluation

The spreadability, homogeneity, sensory characteristics, and pH tests showed that all formulas met the evaluation standards. However, F3 had a better SPF value than the other types, so it was used for the advanced evaluation.

3.8.1. Phytochemical Screening

Phytochemical screening is a qualitative test used to identify bioactive compounds in a sample. Based on the

Table 6. Results of the mangrove leaf extract cream sensory test

Formula	Color	Aroma	Texture
F0	White	Cream aroma	Smooth
F1 (2.5%)	Light green	Cream aroma	Smooth
F2 (5%)	Moderate green	Cream aroma	Smooth
F3 (7.5%)	Dark green	Cream aroma	Smooth

Table 7. pH and SPF value of mangrove leaf extract cream

Formulation	pH	SPF
F0 (0%)	7.33	0.673
F1 (2.5%)	7.30	3.901
F2 (5%)	7.14	4.877
F3 (7.5%)	7.03	6.983

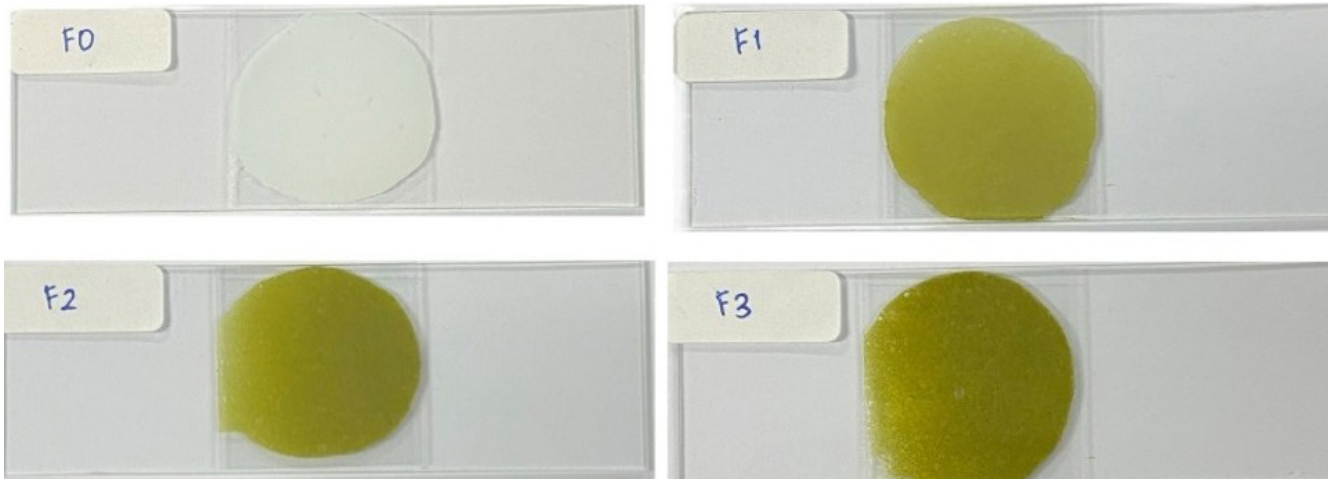


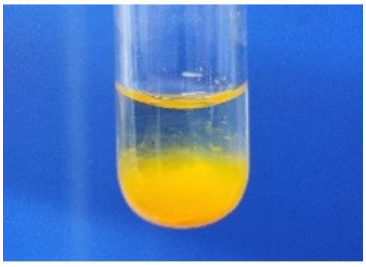
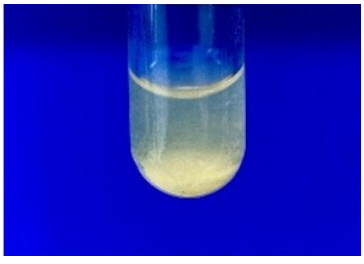
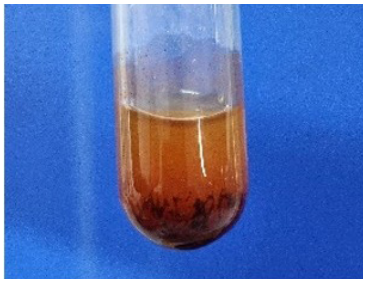
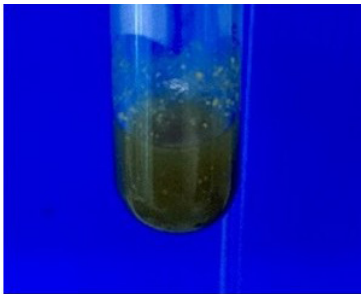
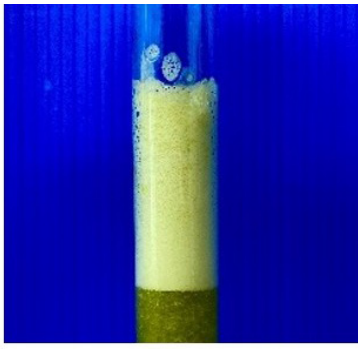
Figure 1. Cream homogeneity test results (F0: plain, F1: 2.5% extract, F2: 5% extract, and F3: 7.5% extract)

phytochemical results, the mangrove leaf extract cream positively contains alkaloids, tannins, and saponins (Table 8).

3.8.2. Centrifugal Test Results

The centrifugal test is one indicator of the physical stability of the semi-solid cream. The results showed

Table 8. Phytochemical screening results of mangrove leaf extract cream

Type	Result	Documentation	Standard
Alkaloid: Dragendorff	Positive		An orange precipitation
Mayer	Positive		A creamish or pale yellow precipitation
Wagner	Positive		A reddish-brown precipitation
Tannin	Positive		Blackish-green color
Saponin	Positive		The formation of foam

no change in color or aroma after the centrifugal test, which required minimal stirring to mix the phases. The results of the cream pH after the centrifugal test increased compared to the before but still was in the neutral range, with a pH value of around 7.

The results of the microbial contamination test revealed that no microbial colonies were found in the mangrove leaf extract cream before the centrifugal test. Only a few microbes were found after testing. Most of the microbes were found after the centrifugal test in the 10^{-1} dilution, with a total of 26 (Table 9).

3.8.3. Histological Analysis Results

3.8.3.1. Mean Epidermal Thickness

The results revealed that the mean epidermal thickness of mice in plain cream treatment (P1) and extract cream treatment (P2) was higher than the control (C). Based on the One-way ANOVA test, a significance value of 0.001 was obtained, so there was a significant difference between the cream treatment and control groups.

Further analysis of the mean epidermal thickness differences among groups proceeded with the LSD test. The results showed that P2 significantly differed from C and P1 ($p<0.05$). C and P1 did not significantly differ ($p>0.05$).

The lowest neutrophil count was in P2. Based on the statistical test, the neutrophil count in P2 significantly differed from P1 and C ($p<0.05$). The highest mean fibroblast count was in P2 (18.24±0.75) compared to P1 and C. Based on the statistical tests, the number of fibroblasts in P2 significantly differed from P1 and C ($p<0.05$). The descriptive data of the mean epidermal thickness, neutrophil counts, and fibroblast can be seen in Table 10. The visualization of the epidermal thickness in Figure 2.

Table 9. pH value and microbial test results before and after the centrifugal test

Treatment	Dilution	Total microbes
Before the centrifugal test (pH = 7.03)	10^{-1}	0
	10^{-2}	0
After the centrifugal test (pH = 7.17)	10^{-1}	26
	10^{-2}	1

4. Discussion

The antibacterial test showed that *A. marina* exhibited the largest inhibition against the growth of *P. aeruginosa* and *S. aureus* (11.68±0.25 mm and 11.33±0.10 mm, respectively). The antifungal test showed the largest inhibition against *C. albicans* by *A. marina* (9.65±1.22 mm) and against *M. furfur* by *R. mucronata* (7.65±0.78 mm). However, all extracts used in the study had antibacterial and antifungal activity. These findings are consistent with several studies showing mangrove extracts can inhibit the growth of pathogenic bacteria and fungi (Audah *et al.* 2018; Ibrahim *et al.* 2022).

The antioxidant and photoprotective activity test revealed that *A. marina* had the highest effect, with a percentage inhibition of 50.15% and SPF of 3.348. The percentage of free radical inhibition increases with the antioxidant compounds, which combat free radicals (Iranawati *et al.* 2020). According to Lopes *et al.* (2022), variations of bioactive compounds like

Table 10. Descriptive data of the mean epidermal thickness, neutrophil counts, and fibroblast of mouse skin

Treatment	Epidermal thickness (μm)	Neutrophil	Fibroblast
C (Control)	19.48±4.16^{a}	35.32±1.40^{a}	10.48±0.81^{a}
P1 (Plain cream)	25.32±2.44^{a}	31.16±1.01^{b}	11.80±0.51^{b}
P2 (Extract cream)	38.52±7.83^{b}	14.16±0.79^{c}	18.24±0.75^{c}

Different superscript letters per measurement parameter indicate significant differences at the 95% confidence level

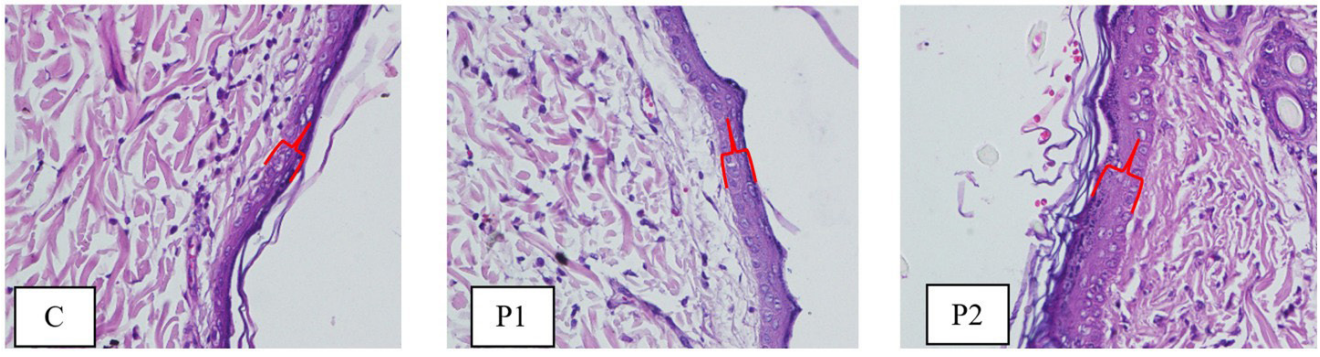


Figure 2. Visualization of the epidermal thickness of mouse skin in the three groups (C: control, P1: plain cream, P2: mangrove leaf extract cream)

flavonoids, anthocyanins, condensed tannins, and biogenic amines affect SPF values by increasing ultraviolet light absorption.

The spreadability of the cream in this study conformed to the standard for good cream spreadability, which was between 4-7 cm. This range suggests a semisolid form that is comfortable for users (Garg *et al.* 2002; Sapiun *et al.* 2022). Sapiun *et al.* (2022) indicated that the concentration of extract directly affects the spreadability, with higher extract concentrations improving spreadability. Good spreadability enhances the contact area between the active substance and the skin, allowing rapid absorption. The homogeneity test showed that the cream's quality was good, as the active substance was uniformly distributed. Inhomogeneous cream may result in reduced therapeutic efficacy. The pH value adhered to the standard for sunscreen, which was between 4.5-8 (SNI 16-4399-1996). A significant pH deviation from the skin's physiological pH increases the risk of adverse effects. Low pH can irritate the skin, while high pH can lead to dryness and scaling (Hawkins *et al.* 2021).

The results demonstrated that adding *A. marina* extract influenced the SPF value of the cream, which acts as a photoprotective agent. *A. marina*, especially in Semarang's Tugurejo area, is naturally exposed to sunlight, making it can survive exposure to UV radiation and relatively high temperatures. UV exposure induces the production of secondary metabolites in mangrove leaves, which play a role in mitigating photo inhibition and stabilizing free radicals (Yeshi *et al.* 2022). The cream with the addition of 7.5% extract has the highest SPF value of 6.98. Table 9 shows that the higher the percentage of extract added, the higher the SPF value due to the increase in bioactive compounds that can absorb ultraviolet light (Lopes *et al.* 2022).

The centrifugal test indicated no changes in the cream's color or aroma, with only minimal stirring required to mix the phases. Zheng *et al.* (2022) stated that emulsifiers in the cream act as surfactants, reducing the interfacial tension between oil and water and preventing phase separation. The microbial contaminant test showed that microbial levels in the cream were safe, with total colony counts below the limit based on SNI 19-2897-1992, with a maximum of 10^2 colonies/gram.

The histological analysis of epidermal thickness showed that the mangrove leaf extract cream inhibited the thinning of the epidermis in UV-exposed mice. UV-induced skin aging, known as photoaging, causes various epidermal cells, such as keratinocytes, melanocytes, mast cells, and Langerhans cells in the stratum spinosum and granulosum layers, to decrease and impact the epidermal thickness (Ansary *et al.* 2021). Lee *et al.* (2021) stated that the skin could renew through cell turnover. New cells are formed in the stratum basale and gradually travel up to the outermost layer of the epidermis to replace dead or damaged cells. However, the epidermal cell cycle prolongs when the skin ages, leading to thinner skin.

In neutrophil analysis, group C showed the highest count due to UV-B-induced skin inflammation, which triggers a rapid migration of white blood cells to the affected area. Neutrophil infiltration peaks around 24 h after UV-B exposure (Neale *et al.* 2023), drawn by inflammatory cytokines such as IL-8, LTB-4, and TNF-α (Maddipati 2020). This study found that using mangrove leaf extract cream significantly reduced the number of neutrophils, suggesting its anti-inflammatory activity. The results of this study are in line with Li *et al.* (2018), which state that the application of topical extract cream containing alkaloid, flavonoid, tannin, triterpene, and saponin, can reduce TNF-α levels, thereby reducing neutrophils and inflammation.

UV-B irradiation can activate inflammatory pathways and induce oxidative stress, which can cause fibroblast apoptosis. Fibroblasts are the most common cell type that synthesize collagen, a protein that provides skin elasticity and strength. UV irradiation can interfere with collagen synthesis by fibroblasts and induce the expression of matrix metalloproteinase (MMP) protein, which degrades collagen and elastin, reducing skin elasticity and causing skin wrinkles (Nasti & Lubis 2023).

Fibroblast analysis showed that mangrove leaf extract cream preserved fibroblast levels, preventing their decrease in UV-exposed skin. This result aligns with Khan *et al.* (2012), who found that plant extracts can protect fibroblasts and keratinocytes from UV-B damage by preventing mutations in the mitogen-activated protein kinase (MAPK) and MMP proteins and nuclear factor kappa B (NF-KB) degradation.

The phytochemical screening showed that the cream contained alkaloids, tannins, and saponins. Alkaloids act as antioxidants by donating electron pairs, reducing free radical activity (Mahani *et al.* 2022). Alkaloids play a role in inhibiting microbial cell wall synthesis, compromising cell wall integrity, disrupting cell membrane permeability, inhibiting microbial metabolism, and inhibiting nucleic acid and protein

synthesis (Yan *et al.* 2021). Tannins promote epidermal growth, heal wounds, and re-epithelialize cells by precipitating lipid proteins (Singh & Kumar 2019). Research by Khan *et al.* (2012) revealed that tannins act as photoprotectors by inhibiting UV-B rays and further preventing the formation of MAPKs, MMPs, translocation, and phosphorylation of NF-KB/p65, as well as inhibiting free radical-induced DNA mutations. Saponins can help synthesize collagen and cell-matrix proteins, thereby preventing aging (Demirbas 2021).

The efficacy of *A. marina* leaf extract in inhibiting skin pathogenic microorganisms and its antioxidant and photoprotective capacity make it a substantial candidate as a natural raw material for the cosmetics industry. Research has shown that herbal formulations and extracts as natural ingredients have consistent quality and can be easily used in cosmetic creams. Therefore, *A. marina* leaf extract presents promising potential for development as a natural product for the cosmetics industry.

In conclusion, the results showed that mangrove leaves of *R. mucronata, A. marina*, and *B. gymnorrhiza* species exhibit potential as antibacterial, antifungal, antioxidant, and photoprotective agents with the best overall bioactivity in *A. marina* species. The cream formulated with the extract contained alkaloids, tannins, and saponins. The cream was homogeneous, as the active ingredients were evenly distributed. It also displayed good physical stability, with no changes in color and aroma after the centrifugal test. Both the pH value and total microbes of the cream, before and after the centrifugal test, complied with SNI standards. The histological analysis showed that the cream effectively prevented epidermal thinning, reduced neutrophil count, and maintained fibroblast number in UV-B exposed mouse skin compared to untreated skin.

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