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Sun Protection Factor (SPF) Activity of Lime Peel Extract (*Citrus aurantifolia*) on Mice (*Mus musculus*)

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ABSTRACT

***Introduction**: Excessive ultraviolet (UV) exposure causes various skin problems such as erythema and pigmentation. Lime peel extract (Citrus aurantifolia) contains flavonoids that have the potential to act as natural sunscreen agents because of their ability to absorb UV rays. **Aims**: This study aims to evaluate the Sun Protection Factor (SPF) activity of lime peel extract in cream form and to determine its effectiveness in protecting the skin from UV-B radiation. **Methods**: The extract is obtained by maceration using 70% ethanol and formulated into creams with concentrations of 6%, 10%, and 12%. The in vivo SPF activity test is carried out on mice (Mus musculus) exposed to UV-B light, and SPF values are measured by the Mansur method using UV-Vis spectrophotometry. **Result**: Phytochemical screening shows the presence of flavonoids, phenolics, tannins, and saponins. The 10% and 12% cream formulations show average SPF values of 25.5 and 27.6, which belong to the medium protection category, and both prevent the appearance of erythema and edema on the mice's skin. Statistical analysis using One-Way ANOVA shows a significant difference ($p < 0.05$) among the formulations, indicating that increasing the concentration of lime peel extract improves UV protection. **Conclusion**: The results show that lime peel extract has sunscreen activity comparable to commercial sunscreen with SPF 35 and has potential as a natural photoprotective agent.*

***KEYWORDS**: Lime peel, ultraviolet light, sunscreen, SPF, mice*

INTRODUCTION

Indonesia is exposed to direct sunlight with high intensity throughout the year, causing the surrounding areas to have high air temperatures. Ultraviolet rays are part of the electromagnetic spectrum that comes from the sun. Prolonged exposure to UV rays on the skin without protection can cause various negative effects. Exposure to UV radiation can damage skin cell DNA and also cause a burning sensation on the skin (sunburn). Other effects include contact dermatitis, decreased immune system function, loss of skin elasticity, wrinkles, edema, pain, and skin conditions such as photodermatosis (Fitraneti *et al*., 2024).

Products that protect the skin from sun exposure are known as sunscreens. The

effectiveness of sunscreen protection is measured by the SPF (Sun Protection Factor), which indicates how well the product protects the skin from ultraviolet radiation (Stanfield., 2003 in Anggreini *et al*., 2024). The higher the SPF number, the greater the sunscreen's ability to protect the skin from ultraviolet radiation (Baki., 2015 in Anggreini *et al*., 2024).

One natural ingredient with potential as an active ingredient in sunscreen products is lime peel (*Citrus aurantifolia*). Lime peel is often discarded as waste because it is not used in the production of juice, food, or other uses. However, lime peel waste can be processed to obtain its compounds, which have many benefits, one of which is as a sunscreen (Kurniandari *et al*., 2015 in Pratikto *et al*., 2024).

Previous research has shown that ethanol extracts from lime peel contain flavonoids with strong antioxidant activity. Compared to other parts of the plant, such as the fruit and leaves. Lime peel has a higher total phenol and flavonoid content (Wardiyah *et al*., 2022). The flavonoid compounds in lime peel have the potential to be used as sunscreen ingredients because they contain chromophore groups. These chromophore groups are able to strongly absorb ultraviolet rays in the UVA and UVB wavelength ranges, thanks to their conjugated aromatic system (Suryadi *et al*., 2021).

Based on the above description, the researchers were interested in testing the activity of lime peel extract (*Citrus aurantifolia*) in protecting mice skin from ultraviolet light exposure.

METHODS

Materials and Instruments

The materials to be used are distilled water, stearic acid, 70% ethanol, FeCl3, glycerin, concentrated HCl, lime peel (*Citrus aurantifolia*), filter paper, lanolin, methyl paraben, propyl paraben, cetyl alcohol, and TEA.

The tools to be used are a stirring rod, beaker, porcelain dish, glass funnel, chemical glass, measuring cup, furing cloth, UV lamp (exotera lamp), oven, dropper, water bath, rotary evaporator, spatula, test tube, analytical scale, and digital scale.

Sample Prepration and Extract Preparation

Samples of lime fruit (*Citrus aurantifolia*) that had been collected were washed using running water, then the peel and flesh were separated and chopped. The lime peel pieces were dried by airing them, then ground into powder. After that, the lime peel powder was weighed (Auliasari & Siarumtias, 2020).

The extraction method used was maceration. Maceration was carried out by soaking 250 grams of dried lime peel powder in 70% ethanol solvent using a

Table 1. Lime peel extract cream formula.

Ingridients	Function	Formula (%)			
		F0	F1	F2	F3
Lime Peel Extract	Active Ingredient	-	6	10	12
Stearic Acid	Emulsifier	8	8	8	8
Cetyl Alcohol	Stabilizer	6	6	6	6
Lanolin	Emollient	1	1	1	1
TEA	Emulsifier	1	1	1	1
Gliserin	Humectant	10	10	10	10
Methyl Paraben	Preservative	0,18	0,18	0,18	0,18
Propyl Paraben	Preservative	0,02	0,02	0,02	0,02
Aquadest (ad)	Solvent	100	100	100	100

macerator. After extraction, the collected extract was then concentrated by air drying using a fan until a thick extract was obtained (Cahyani & Widiastuti, 2020). Then, the yield was calculated using the following formula:

$$\%\text{Yield} = \frac{\text{Concentrated extract weight (g)}}{\text{Simplified weight (g)}} \text{ x } 100$$

Phytochemical Screening

Flavonoid test

Weigh 100 mg of sample dissolved in 10 mL of ethanol p.a. Then take 1 mL of sample, add 1 mg of Mg powder and add 5 drops of HCl. If red, yellow and orange colors are formed, then it is positive for flavonoids (Sari *et al*., 2024).

Phenolic test

Weigh 100 mg of sample dissolved in 10 mL of p.a. ethanol. Then take 1 mL of sample and add three drops of 1% FeCl3 solution. If a blackish purple color forms, this indicates a positive result for phenolics (Sari *et al*., 2024).

Tannin test

Weigh 100 mg of sample dissolved in 10 mL of p.a. ethanol. Then take 1 mL of sample and add 3 drops of 1% FeCl3. A positive result can be seen based on the formation of a bluish-green or blackish-green color (Sari *et al*., 2024).

Saponin test

Weigh 100 mg of sample and dissolve in 10 mL of ethanol p.a. Take 1 mL of sample and place it in a test tube, then add 5 mL of distilled water and shake for 1 minute. If foam forms, add 4 drops of 1 M HCl solution. If no foam forms, continue heating for 2-3 minutes. Allow to cool, then shake vigorously. The formation of stable foam within 8-10 minutes indicates the presence of saponin compounds in the sample (Sari *et al*., 2024).

Lime Peel Extract Cream Formulation

The cream preparation formula was made using various concentrations of lime peel extract, as shown in the following Table 1.

The oil phase, consisting of stearic acid, cetyl alcohol, lanolin, and propyl paraben, is melted in a water bath at 70°C. Meanwhile, the water phase, which includes TEA, glycerin, and methyl paraben, is also heated to the same temperature together with distilled water. The cream is made by pouring the water phase into a heated mortar, then slowly adding the oil phase while stirring until a creamy mass is formed. After that, lime peel extract is added and stirred until the mixture becomes homogeneous (Pratikto *et al*., 2024).

Determination of SPF value

The SPF value was determined using the UV-Vis spectrophotometry method. A total of 0.1 grams of sample, either in the form of preparations or extracts of lime fruit peel (*Citrus aurantifolia*), was dissolved in 10 mL of 70% ethanol to produce a stock solution with a concentration of 30,000 ppm. The solution was then gradually diluted to obtain concentrations of 4,300 ppm, 4,400 ppm, and 4,500 ppm. Next, the absorbance of each solution was measured at wavelengths of 290–320 nm at 5 nm intervals using a UV-Vis spectrophotometer (Octaviani, 2023). Each formula was measured three times, and the absorbance values obtained were calculated using Mansur's formula:

$$\text{SPF} = \text{CF X} \sum_{290}^{320} \text{EE } (\lambda)\text{I } (\lambda) \text{ X Abs } (\lambda)$$

Where EE are erythemal effect spectrum, I are solar intensity spectrum, Abs are absorbance of sunscreen product, and CF are correction factor.

Testing the protective ability of sunscreen on mice

A total of 15 mice aged 2–3 months with a body weight of 25–30 grams were used as test animals. Before treatment, the mice were acclimatized for 5 days. The animals were then divided into 5 groups, each consisting of 3 mice. The positive control group was given commercial sunscreen with SPF 30, while the negative control was given a cream base without active ingredients. Group F1 received cream with a 6% lime peel extract combination, group F2 received a 10% concentration, and group F3 received a 12% concentration. Before treatment, the fur on the mice's backs was shaved to form an area measuring 3×4 cm. The area was then coated with the lime peel extract cream preparation and left for 1 hour. After that, each group of mice was exposed to light from an Exotera lamp for 48 hours. Observations of skin reactions were made after 24 hours and 48 hours, with assessments using a 0–4 scale based on skin surface conditions (Indarto *et al*., 2022).

The erythema and edema scores obtained were analyzed to calculate the Primary Dermal Irritation Index using the following formula:

$$\frac{A + B}{C \; x \; t}$$

Where A are total erythema score at the time of observation, B are total Edema score at the time of observation, C are number of mice, and t are number of observation times.

RESULTS AND DISCUSSION

Extraction Results from Lime Fruit Peel

The yield from the extraction process of lime peel (*Citrus aurantifolia*). From 500 grams of dried lime peel used, a thick extract weighing 37.8 grams was obtained. Thus, a yield of 7.56% was obtained (Table 2). This value indicates that the extraction process used is quite efficient in extracting active compounds from natural materials. However, the yield was not in accordance with the herbal pharmacopoeia, which stipulates that the yield of lime peel extract should not be less than 15.0% (Kementerian Kesehatan RI, 2017). This was due to the fact that the remaceration process was only carried out once, so that the extraction of active compounds from the crude material was not optimal and some of the content was not extracted to the maximum extent possible.

Phytochemical Screening of Lime Peel Extract

The phytochemical screening results showed that lime peel extract (*Citrus aurantifolia*) contains secondary metabolites in the form of flavonoids, phenolics, saponins, and tannins (Table 3). The flavonoid content was indicated by the formation of an orange color after the addition of Mg and concentrated HCl, indicating a positive result. Phenolic compounds were detected through a reaction with $FeCl_3$, which produced a blackish brown color. The saponin test showed positive results with the formation of stable foam for 8–10 minutes, while the tannin test gave positive results with the formation of a blackish green color after the addition of $FeCl_3$.

These results are consistent with previous studies (Auliasari & Siarumtias, 2020; Cahyani & Widiastuti, 2020; Suryadi *et al*., 2021; Wardiyah *et al*., 2022), which also reported that lime peel contains flavonoids, phenolics, saponins, and tannins. The presence of these compounds plays an important role in the biological activity of the extract, especially flavonoids and phenolics, which are known to have antioxidant and UV radiation protection properties, thus supporting the potential of

Table 2. Percentage yield of lime peel extract

Simplisia weight (g)	Extract weight (g)	% Yield
500	37.8	7.56

Table 3. Phytochemical screening results

Secondary metabolites	Results
Flavonoids	+
Phenolics	+
Saponins	+
Tannins	+

Table 4. The potential value of lime peel extract as a sunscreen

Formula	SPV (ppm)			Protection	One-way ANOVA results (sig)
	4.300	4.400	4.500		
F0	-0.4	-0.3	-0.1	No protection	
F1	11.6	12.8	13	Low	0.000
F2	25.5	25.7	25.4	Moderate	($p < 0.05$)
F3	27.5	27.9	27.6	Moderate	

Table 5. Erythema and edema irritation index

Test group	Effect		Iritation indeks	One-way ANOVA results (sig)
	Eritema	Edema		
F0	✓	✓	0.02	
F1	✓	-	0.01	0.001
F2	-	-	0	($p < 0.05$)
F3	-	-	0	
F4	-	-	0	

lime peel extract as an active ingredient in natural sunscreen.

Potential Value of Lime Peel Extract Sunscreen

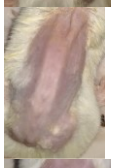
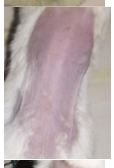
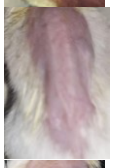
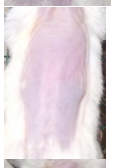
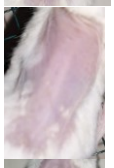
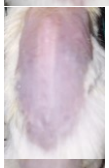
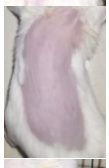
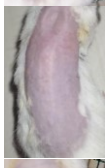
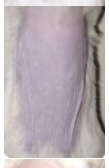
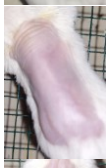
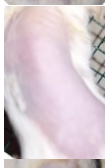
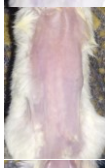
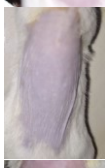
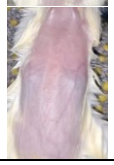
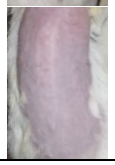
The results (Table 4) show that F0 or cream without extract has a negative SPF value (-0.4 to -0.1), which means it does not provide protection against UV rays. Meanwhile, F1 with 6% extract shows an SPF value between 11.6–13, which is classified as low protection. In F2 (10%), the SPF value increased dramatically to 25.4–25.7, falling into the moderate protection category. F3 with 12% extract showed the highest SPF value, namely 27.5–27.9, also classified as moderate protection.

Based on the results of the One Way ANOVA test, a significance value of 0.000 ($p < 0.05$) was obtained, indicating that variations in extract concentration have a very significant effect on the SPF value. This reinforces that an increase in lime peel extract concentration is directly proportional to an increase in the effectiveness of protection against UV rays. The concentration of extract added to the cream formula significantly affects the absorption of ultraviolet rays (Fajari, 2022).

Observations on The Backs of Mice

Based on the observations listed in Tables 5 and 6, it can be seen that the negative control group (F0), which was only given a cream base without the addition of active lime peel extract, experienced erythema and edema at 24 hours, with conditions worsening at 48 hours. This condition was caused by exposure to ultraviolet B (UVB) rays, which are known to cause reactions such as redness of the skin (erythema) and swelling (edema). These symptoms generally begin to appear 2 to 3 hours after exposure, with maximum intensity occurring around 10 to 12 hours later (Adzhani *et al.*, 2022).

Table 6. Observation on the back of mice

Test group		After irradiation		
		1	2	3
F0	24 hours			
	48 hours			
F1	24 hours			
	48 hours			
F2	24 hours			
	48 hours			
F3	24 hours			
	48 hours			
F4	24 hours			
	48 hours			

A similar result was observed in the formulation with a concentration of 6% (F1), which still caused irritation reactions in the form of mild erythema and edema in mice 1 and 3. Meanwhile, no erythema or edema was observed in mice 2. These differences in reaction may be due to biological variability between individuals,

which is common in the use of laboratory animals. Factors such as skin sensitivity, epidermal thickness, melanin content, and local immune resistance of each mice can affect the response to UV exposure and to the active ingredients in the formulation. Meanwhile, in the positive control group (F+) and formulations F2 and F3, no signs of erythema or edema were found, indicating that cream preparations containing active ingredients at concentrations of 10% and 12% were able to provide optimal protection against irritation due to UV exposure (Indarto *et al*., 2022).

The results of the irritation index analysis tested using the one-way ANOVA method showed a significant difference between treatment groups, with a significance value (Sig.) of 0.001. A significance value of less than 0.05 indicates that there is a significant difference in the average irritation index between test groups (Utari *et al*., 2025).

CONCLUSION

Based on the results of the research conducted, it can be concluded that lime peel extract (*Citrus aurantifolia*) contains secondary metabolites such as flavonoids, phenolics, tannins, and saponins that play a role in protecting against ultraviolet (UV) rays. The lime peel extract cream formula with concentrations of 10% and 12% showed SPF values of 25.5 and 27.6, respectively, which are classified as moderate protection and are capable of providing protection against UV-B exposure. Observations on mice skin (Mus musculus) showed that the cream formulations with concentrations of 10% and 12% did not cause skin irritation in the form of erythema and edema, and showed activity comparable to commercial sunscreen with an SPF of 35.

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