

EFFECTIVENESS OF FRANGIPANI LEAF EXTRACT (*Plumeria alba* LINN.) AS A UV FILTER

[Uji Efektivitas Ekstrak Daun Kamboja Putih (*Plumeria alba* Linn.) sebagai UV Filter]

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ABSTRAK

Paparan sinar matahari mengandung sinar UV yang berbahaya bagi kulit, untuk itu diperlukan UV filter. Beberapa UV filter dalam tabir surya komersil menimbulkan dampak berbahaya. Oleh karena itu, diperlukan alternatif UV filter alami yang mengandung flavonoid, salah satunya adalah Daun Kamboja (*Plumeria alba* Linn.). Penelitian ini bertujuan untuk menguji kemampuan flavonoid sebagai UV filter secara *in vitro* dan *in vivo*. Ekstraksi dilakukan dengan maserasi menggunakan etanol 70% dan purifikasi flavonoid digunakan metode cair-cair. Parameter yang diukur adalah total kandungan flavonoid, nilai Sun Protector Factor [SPF], dan skoring gejala klinis penghambatan melanogenesis pada tikus. Rendemen hasil purifikasi sebesar 43,6%. Kandungan flavonoid pada hasil purifikasi (64,4 mg QE/g), lebih tinggi dibanding ekstrak kasar (57,6 mg QE/g). Nilai SPF tertinggi (33,88) ekstrak hasil purifikasi 1400mg/mL, sedangkan pada konsentrasi yang sama ekstrak kasar menghasilkan nilai SPF yang lebih rendah (33,06). Analisis data korelasi Pearson (0,563) menunjukkan adanya hubungan positif yang berbanding lurus antara nilai SPF dan konsentrasi ekstrak. Pengujian *in vivo* dilakukan dengan skoring gejala klinis dimulai dari angka 0-4 berdasarkan tingkat keparahan eritema yang terjadi. Setelah 21 hari paparan sinar matahari, kelompok yang mengalami eritema terparah adalah kelompok kontrol negatif (skor 1,292), sedangkan hasil terbaik adalah kelompok perlakuan pengolesan ekstrak purifikasi 1400mg/mL (skor 0,542).

Kata Kunci: flavonoid, *Plumeria alba*, skoring eritema, SPF, dan UV filter.

ABSTRACT

UV filters are necessary since UV rays are harmful to human skin. UV filter compounds in commercial sunscreen products can cause adverse effects. Thus, natural UV filters containing flavonoids, e.g. Frangipani (*Plumeria alba* Linn.) leaves, are needed. This research examined the use of flavonoids from *P. alba* leaf as a UV filter with *in vitro* and *in vivo*. Maceration was performed with 70% ethyl alcohol and liquid-liquid extraction for flavonoid purification. Parameters tested were total flavonoid compounds, SPF value, and clinical symptoms of the melanogenesis inhibition scoring. The purification result was 43.6%. The flavonoid concentration in pure extract (64.4 mg QE /g) was higher than crude extract (57.6 mg QE/g). The highest SPF value (33.88) was observed in pure extract at 1400 mg/mL, while the same concentration of crude extract resulted in a lower SPF value (33.06). Pearson correlation analysis (0.536) indicated a directly proportional relationship between SPF value and extract concentration. *In vivo* analysis was performed by scoring between 0-4 following the severity of erythema. After 21 days of sunlight exposure, the worst erythema was found in the negative control group (score 1.292). The best result was found in the group treated with pure extract 1400 mg/ml (score 0.542).

Keywords: flavonoid, scoring erythema, *Plumeria alba*, SPF, and UV filter.

INTRODUCTION

Based on the wavelength, UV radiation can be divided into UVA (315–400 nm), UVB (280–320 nm), and UVC (100–280 nm). Among the three types of UV light, only UVA and UVB can penetrate through the Stratosphere. About 90–95% of incoming UV light is UVA which plays a role in the occurrence of “aging”, while the rest is UVB which plays a role in occurrence of erythema and skin cancer (Dorazio *et al.*, 2013; Tampucci, 2017). UVB rays are more harmful than UVA because it damages the skin directly (Naranayan *et al.*, 2010). To avoid the adverse effects of UV radiation, skin protectors such as sunscreens are needed.

Sunscreens can absorb at least 85% of the sun rays at the wavelengths from 290 to 320 nm for UVB but wavelengths more than 320 nm for UVA

are not absorbed (Suryanto, 2012 in Alhabsy *et al.*, 2014). Based on their origin and capabilities, sunscreens are divided into organic UV filters (chemical) and inorganic UV filters (physical). Organic UV filters work by absorbing UV radiation then turning it into lower energy so that it does not cause sunburn effects on the skin, whereas inorganic UV filters work by reflecting or blocking UV rays (Lavi, 2012; Rosyidi *et al.*, 2018). Benzophenone-3, avobenzone, methoxycinnamic, and octylsalicylate are used as organic UV filters (Rosyidi *et al.*, 2018). Flavonoid can act as ultraviolet induction radicals (UV), by absorbing the UV rays. Some plants containing flavonoid and phenolic compounds have antioxidant benefits and are also known to be used as sunscreens (Fonseca, 2015; Pradika, 2016).

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Frangipani (*Plumeria alba* L.) is a plant containing flavonoids. Dawood *et al.* (2016) have shown that the leaf of *P. alba* contain flavonoids like quercetin and rutin which have been reported to act as UV filters (Choquenot *et al.*, 2008; Marcos *et al.*, 2016). Frangipani plants are widely distributed in Indonesia and can grow and reproduce without requiring special treatment (Wrasiati *et al.*, 2011). Frangipani plants have multiple varieties, one of which is white frangipani (*P. alba*).

Frangipani leaf extract has been utilised as an anti bacterial against *Staphylococcus aureus* (Ningsih *et al.*, 2014), *P. alba* flower essential oil as mosquito repellent aroma therapy (Nurcahyo and Purgiyanti, 2017), and *P. alba* flower extract as an anti-bacterial against *S. aureus* and *Salmonella typhi* (Rupianasih *et al.*, 2019). Based on several existing studies, the flower is the most commonly utilized component of the plant, while the leaves have seen little research. Seeing the potential of *P. alba* leaf, this study aimed to test the effective ness of flavonoids from Frangipani leaf extract as UV filters on the skin of Wistar rats that exposed to sunlight for 21 days.

MATERIALS AND METHODS

Tools and materials

Test tube, micropipette, blender, oven, rotary vacuum evaporator, UV-Vis Spectrophotometer, a set of surgical instruments (surgical scissors and scalpel), 100 mesh sieve, 250mL separating funnel, water bath, and filter cloth.

Plumeria alba leaves were sampled from housing complexes around Kalasan, Sleman, Yogyakarta. The wet weight of *P. alba* leaf used was 7 kg. Male Wistar rats aged 9 weeks weighing 150–200 g were used as test animals on *in vivo* SPF value testing, Aquadest, Aluminium chloride (AlCl_3) 10%, Ethanol 70%, *n*-Hexane, Potassium Acetate (CH_3COOK) 1M, and methanol pro ana-lyse (p.a).

Sample preparation

Plumeria alba leaves washed, removed the major veins, sliced (± 1 cm), and dried in an oven at 60°C for ± 5 days. The dried leaves were then blended and sifted with a 100 mesh sieve. The resulting sample was stored in a plastic container with the addition of silica gel sachets to keep the sample in a dry state.

Extraction

Plumeria alba leaf extraction was carried out using a maceration method, with ethanol 70% as solvent. A total of 1.573 g dried *P. alba* was put into a glass jar and 70% ethanol was added until the powder was covered to a height of 3 cm. The

extraction process lasted for 5 days at 25°C with the jar closed, protected from sunlight, and stirred for the first 24 hours. The extract was filtered using filter cloth to separate liquid from the remaining leaf debris. The filtrate was then evaporated at 40°C for ± 4 hours, until the extract became thick, to produce the crude extract.

Purification extract

Purification of the *P. alba* leaf extract was done to obtain the pure extract which contains flavonoids as target metabolic compounds. Purification was carried out by liquid-liquid extraction method based on Kusnadi and Devi (2017). A total of 50 g of crude extract was placed in separating funnels with 30 mL *n*-hexane as the solvent, then shaking it until the two-phase separation occurred. The upper phase is the *n*-hexane solution with the non-polar compounds from the extract, while the lower phase contains the polar compounds in the extract such as flavonoids. The lower phase was then collected on a porcelain cup and evaporated in the water bath at 40°C until it thickened. This was then categorized as a pure extract.

Determination of the quantity of flavonoid content

Determination of the quantity of Flavonoid content in samples was based on a standard curve that uses pure quercetin as a standard solution. Standard solutions are made with ethanol 70% as solvent and concentrations of 40, 50, 60, 70, 80, 90, and 100 ppm quercetin. The concentration of Flavonoids was analyzed in both crude and pure extract. 1 mL of each sample was analyzed with the addition of 0.2mL of 10% AlCl_3 , 0.2 M of Potassium acetate, and adding up to 10 mL of distilled water. After 30 minutes, the absorbance of the sample was measured at a wavelength of 431 nm.

Determination of in vitro SPF value

In vitro SPF measurements were carried out to test the effectiveness of *P. alba* leaf extract as a UV filter before being tested on animals (*in vivo*). SPF value measurements were carried out using a spectrophotometer at a wavelength of 290–340 nm with intervals of 5 nm. The pure and crude extracts were first diluted in 70% ethanol solution to the concentration of 600 mg/mL, 800 mg/mL, 1000 mg/mL, 1200 mg/mL, and 1400 mg/mL. The SPF value was determined using the Mansur *et al.* (1986) formulation (Sayre *et al.* (1979).

*CF = Correction Factor (10), EE = Erythral Effect of Radiation, I = Solar Intensity Spectrum.

Determination of *P. alba* leaf extract effectivity

as a UV filter (in vivo testing)

In vivo testing was carried out to test the effectiveness of *P. alba* leaf extract as a UV filter by looking at the severity of the erythema experienced by testing animals. Ethical clearance (1174/C.16/FK/2020) was obtained prior to testing. The animals used were male wistar rats aged 8–12 months. Test animals were randomly divided into three groups: positive control (group A), negative control (group B) and treatment group (group C). The negative control group only received the application of distilled water, 30 minutes before irradiation. The positive control group received the

$$SPF = CF \times \frac{290}{320} \sum EE(\lambda) \times I(\lambda) \times \text{absorbance}(\lambda)$$

application of commercial sunscreen with SPF 30, 30 minutes before irradiation. The treatment group received the application of *P. alba* leaf pure extract of 1400 mg/ml. Each group of animals was exposed to sunlight for one hour (11.00–12.00 a.m.) WIB, for 21 days with observations every 3 days. Euthanasia of test animals was carried out on the 22nd day. In vivo testing was done by assigning a score between 0 to 4 in which absence of erythema is scored 0, erythema just visible is scored 1, erythema clearly demarcated is scored 2, moderate to severe erythema is scored 3, and very severe erythema (red beets) with crust formation is scored 4. The final results of each group's scoring were averaged for all individuals and the highest score indicates the worst erythema.

RESULTS**Extraction and purification**

A total of 1573 g sample of *P. alba* leaf with a moisture content of 22.5% were extracted by maceration methods using 70% ethanol as solvents for 5 days resulting the yield of 9.6% (Table 1). Based on the extraction result obtained, purification was carried out as the second stage of extraction to obtain the active compound of flavonoids. The solvent that used in the purification is *n*-hexane as much as 30 ml. Purification result from 90 g crude extract, produced 43.6% yield (table 2).

Determination of flavonoid content

Flavonoid analysis was carried out using quercetin as a standard solution, which is one of the flavonoids group that have the ability as UV protector (Marcos *et al.*, 2017). The linear regression equation obtained was, $y = 0.0031x - 0.0655$ with a value of linearity (R^2), 0.9933 which is close to 1 indicating that the correlation between sample concentration and sample absorbance is ideal. The results of quantitative determination of flavonoids show that the highest concentration in the pure extract was 64.4 mg QE/g, while in the crude extract was 57.6 mg QE/g (table 3).

Table 1. The result of Flavonoid determination of *P. alba* leaf extract (*Hasil pengukuran kadar Flavonoid dari ekstrak daun P. alba*)

Sample (sampel)	Absorbance (Absorbansi) (y)	Mean absorbance (Rata-rata absorbansi) (y)	Total Flavonoid Concentration (Konsentrasi total Flavonoid) (mgQE/g extract)
<i>Crude extract</i> (Ekstrak kasar)	0.112	0.112	57.6
	0.110		
	0.115		
<i>Pure extract</i> (Ekstrak murni)	0.135	0.133	64.4
	0.133		
	0.131		

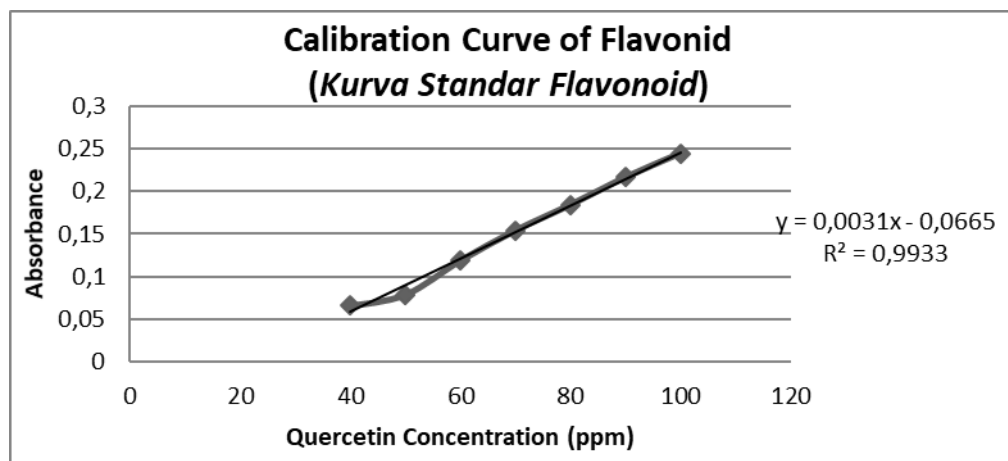


Figure 1. Calibration curve of quercetin in 431 nm wavelength (*Kurva kalibrasi kuersetin pada panjang gelombang 431 nm*)

Determination of *in vitro* SPF value

The SPF value was determined based on Mansur's (1986) equation, by measuring the absorbance at the wavelength of 290 - 320 nm, at 5 nm interval (Sayre *et al.*, 1979). The highest SPF value was obtained in pure extract of 1400 mg/mL, which was 33.88. Extract concentration and SPF value showed a positive correlation both in the crude extract and pure extract as evidenced by the

Pearson correlation value of 0.563 ($p < 0.01$, R^2 0.961, Table 4). Pearson's correlation is considered strong between 0.40 to 0.599 (Kirom, (2015) in Rufaidah *et al.*, (2019)). The R squared (R^2) value of 0.961 means that 96.1% of the SPF value is influenced by differences in concentration and type of extracts, while the 3.9% of the SPF value is influenced by other factors.

Table 2. The result determination of *in vitro* SPF value from *P. alba* leaf extract (*Hasil Pengujian nilai SPF secara in vitro dari ekstrak P. alba*).

No	Concentration (mg/ml)	SPF value		Sunscreen Category
		Crude extract (CE)	Pure extract (PE)	
1	600	8.00	8.42	Extra
2	800	14.16	17.99	Maximal
3	1000	16.15	18.44	Ultra
4	1200	26.37	27.43	Ultra
5	1400	33.06	33.88	Ultra
Pearson Correlation			0.563	
Sig			0.000	
R square (R^2)			0.961	

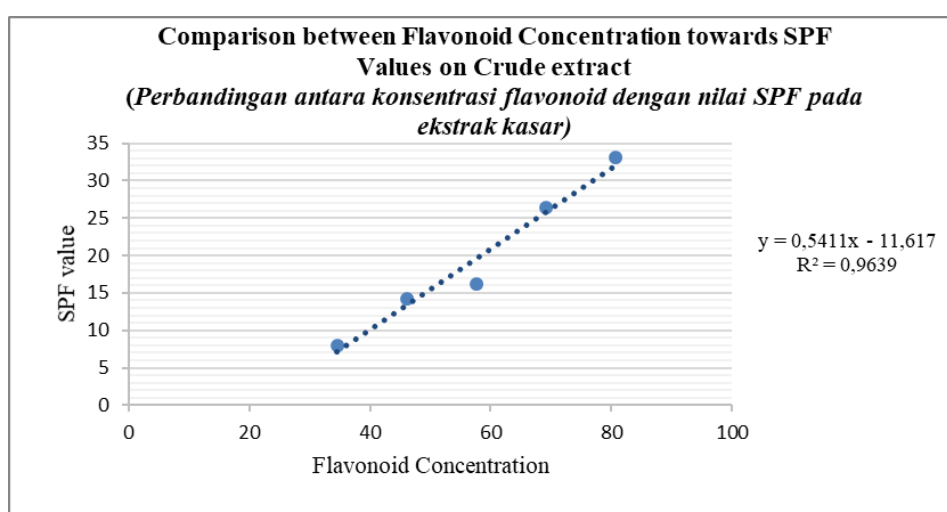
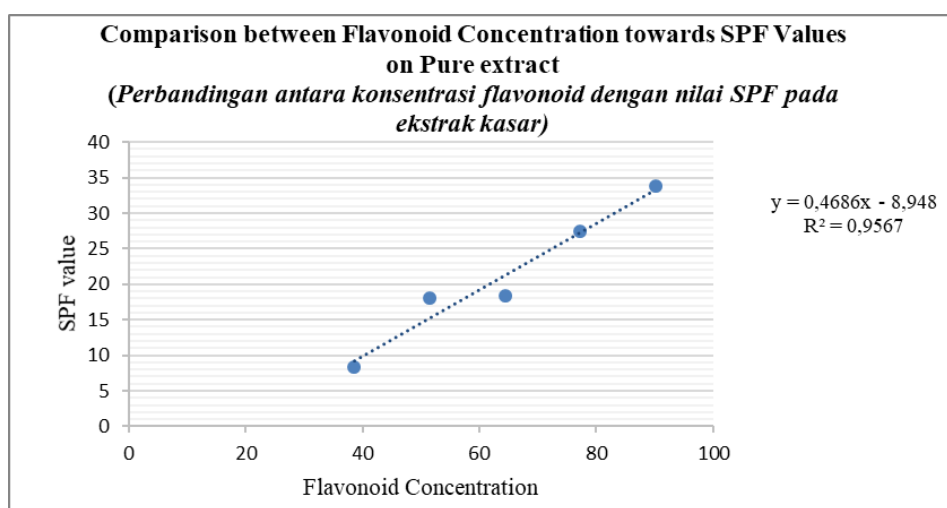
Comparison between Flavonoid Concentration towards SPF Values

Comparison between extract concentration, flavonoid concentration and SPF value is directly proportional where the higher the extract concentra-

tion, the higher the flavonoid content and it also increases the SPF value. Regression value (R^2) obtained shows a good level of confidence because it is close to 1 (Figure 2 and 3).

Table 3. Relationship of flavonoid extract concentration and SPF value
(*Hubungan antara konsentrasi flavonoid dalam ekstrak dan nilai SPF*)

[Extract] [Ekstrak] (mg/mL)	Crude extract (CE) (ekstrakkasar)		Pure extract (PE) (ekstrakmurni)	
	[Flavonoid] (mg)	SPF value	[Flavonoid] (mg)	SPF value
600	34.56	8.00	38.64	8.42
800	46.08	14.16	51.52	17.99
1000	57.6	16.15	64.4	18.44
1200	69.12	26.37	77.28	27.43
1400	80.64	33.06	90.16	33.88

**Figure 2.** Relationship of Flavonoid concentration and SPF value in Crude extract (*Hubungan antara konsentrasi Flavonoid dan nilai SPF pada sampel ekstrak kasar*)**Figure 3.** Relationship of Flavonoid concentration and SPF value in pure extract (*Hubungan antara konsentrasi Flavonoid dan nilai SPF pada ekstrak murni*).

Determination of *P. alba* leaf extract effectivity as a UV filter (in vivo Testing)

Statistical analysis (Anova, R^2 0.964, $p=0.023$) indicates that there is a significant effect of treatment on the severity of erythema in the test animals' skin. The best results are observed in the

test group animals which received the application of pure extract of 1400 mg/mL with the lowest average erythema score (0.542), while the largest erythema score (1.292) was in the negative group of test animals which only received distilled water treatment (Table 6).

Table 4. Average scoring of in vivo testing result on white rats (*Rattus novergicus*) [Skoring rerata hasil pengujian in vivo pada tikus putih (*Rattus novergicus*)].

Days (Hari)	Group of Rat (Kelompok tikus)		
	Group A	Group B	Group C
0	0.000	0.000	0.000
3	0.000	0.000	0.000
6	0.667 ± 0.58	0.000	0.667 ± 1.16
9	1.333 ± 0.58	0.667 ± 1.16	0.667 ± 1.16
12	1.667 ± 0.58	1.667 ± 0.58	0.000
15	1.333 ± 1.16	1.333 ± 1.16	0.667 ± 1.16
18	2.333 ± 0.58	1.333 ± 0.58	1.333 ± 0.58
21	3 ± 0.000	1.333 ± 0.56	1 ± 0.000
Mean	1.292 ± 0.41	0.792 ± 0.48	0.542 ± 0.57
Sig	0.023		
R square (R^2)	0.964		

DISCUSSION

A previous study showed that flavonoid extraction from 200 g of *P. alba* leaf using 70% ethanol as a solvent, and shaken at 120 rpm for 7 days, produced a yield of 6% of dry weight (Ningsih *et al.*, 2014). Ethanol is effective to extract active flavonoids which are polar because they have unsubstituted hydroxyl groups (Kemit *et al.*, 2017). Ethanol 70% was chosen because based on previous researches, it was able to produce the highest yield of flavonoid extraction from the rhizome thatch compared with concentrations of 60%, 80%, and 90% (Arifin *et al.*, 2006; Suhendra *et al.*, 2019). The difference between studies conducted by Ningsih *et al.*, (2014), and this research, indicates that the contact time between sample and solvent effects the yield obtained. The maceration process using a shaker is more optimal than the extraction process with manual stirring. The initial extract from the extraction process is assumed to still have many other active compounds besides flavonoids, so it is called crude extract (CE).

The crude extract was then extracted again through a purification process to get the active flavonoid compounds. Purification was carried out as a second stage of extraction to obtain the flavonoid compounds. The method used in the purification process was liquid-liquid method using separating funnel, based on the process of extracting flavonoids in celery leaf by Kusnadi and Devi (2017). Purification was carried out based on the 'like dissolve like' principles, where the non-polar *n*-hexane solvents will attract the non-polar compounds and two phases will be formed in the separating funnel after shaking out (Figure 4). The upper phase is an *n*-hexane solvent with other non-polar compounds which are still contained in the extract, while the lower phase is a polar compound such as flavonoids (Kusnadi and Devi, 2017). The purification yield is called the pure extract.

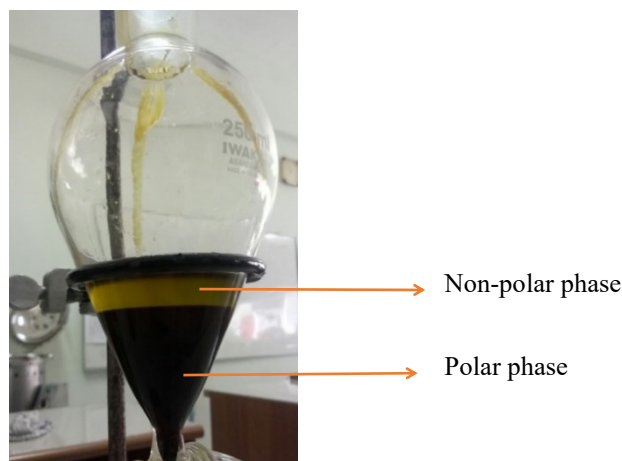


Figure 4. Purification process of *Plumeria alba* leaf extract (*Proses purifikasi dari ekstrak daun Plumeria alba*)

The resulting flavonoid content in pure extract was higher than in the crude extract (Table 3). This showed that flavonoid isolation carried out had separated the flavonoids from other remaining non-polar compounds in the crude extract. Previous studies of flavonoid concentration in frangipani (*P. alba*) leaf have been carried out by Dawood *et al.* (2016), using methanol as a solvent, with a final flavonoid concentration of 74.7 mg QE/g. The difference in this result is possibly due to the different solvent that was used. The higher flavonoid content in the pure extract compared to crude extract was also supported by the SPF value that higher in the pure extract (Table 5). The concentration of extract was shown to have a significant and positive linear relationship with the SPF value evidenced by the positive Pearson correlation value and $p < 0.001$. The highest SPF value was found in the pure extract with the concentration of 1400 mg/mL (SPF 33.88). Based on the categorisation of sunscreen according to the Food and Drug Administration, The SPF value of 33.88 is in ultra-protection category which indicates that the pure extract of 1400 mg/mL has a high ability to protect against UV rays (Ismail *et al.*, 2014). The higher the concentration of the extract used, the higher the concentration of flavonoid it contains. This increases the ability of the extract to function as a UV filter as evidenced by an increase in the SPF value (Table 4). The concentration with the highest SPF value (pure extract, 1400 mg/mL) was used in *in vivo* testing to determine its effectiveness against erythema prevention.

In vivo testing used male *Rattus norvegicus* of the age 8-12 months, to avoid other effects such as the estrogen hormone which plays a role in increasing the amount of melanin in cells and the progesterone hormone which can increase the

spread of melanin in cells (Muller and Rees, 2014). The test was carried out by exposing the rats to the sun for 1 hour per day (11.00 to 12.00 WIB) for 21 days. The treatment was divided into 3 groups namely the positive group which received commercial sunscreen before exposure, the negative group only received distilled water before exposure and the treatment group which received PE extract with a concentration of 1400mg/mL, which had the best SPF value. The application process was carried out 30 minutes before exposure. Observation of test animals was done every 3 days based on the statement of Baumanand Saghari (2009) that pigmentation begins 2-3 days after exposure.

The results revealed that the application of pure extract with a concentration of 1400 mg/mL (group C) produced the lowest score (0.542), followed by the positive control group (group B) that received commercial sunscreen (0.792), and the highest was the negative control group (group A) (1.292). The deviation score between the positive control group and the negative control group was 0.25 which was not very large. It is suggested that the lower scoring of erythema in the treated rat group compared to the positive control was due to the higher SPF value in the extract. In other ways, the extract's color which is black-green also affecting the result, supported by Wilson *et al.* (2018), where dark color such as dark green, red, dark blue and black providing good protection through UV rays, while the darker shades have been associated with the higher UV protection value.

The appearance of rat skin from the positive control group and the treatment group immediately and 30 minutes after application can be seen in Figures 5 and 6. The red circle indicates the application area.

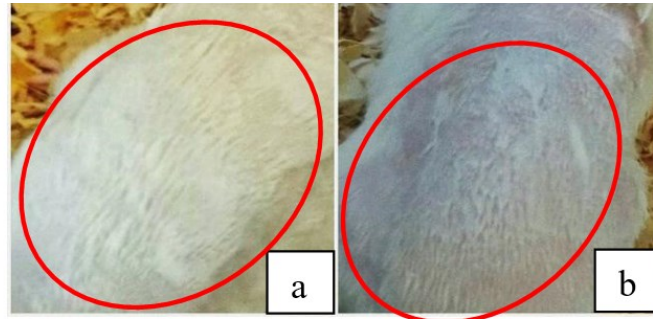


Figure 5. Positive control group (a) after the basting of commercial sunscreen (b) 30 minutes after the basting [Kelompok kontrol positif (a) setelah pengolesan tabir surya komersil (b) 30 menit setelah pengolesan]

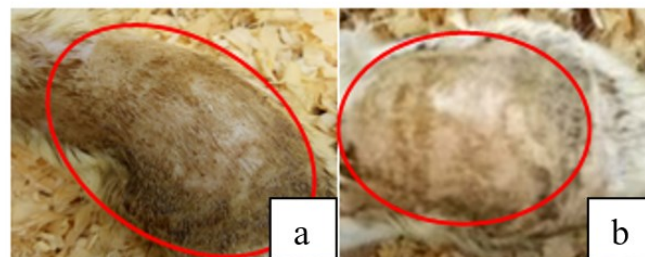


Figure 6. Treatment group (a) after the basting of Pure extract 1400 mg/mL (b) 30 minutes after the basting [Kelompok perlakuan (a) setelah dioles ekstrak murni 1400mg/mL (b) 30 menit setelah pengolesan]

The results of the average erythema scoring value obtained an R square (R^2) value of 0.964 which showed that 96.4% of erythema observed on the skin of test animals was influenced by differences in treatment while the rest (3.6%) was influenced by factors other than differences in the treatment. Some of these factors include the behavior of the rats to lie over each other when exposed to sunlight which causes differences in scores between mice within a group. Weather also

had a large effect on the fluctuation of three daily erythema scoring of the rats. Fluctuation in the score at each observation day indicates that UV filter testing with in vivo method using sun exposure is less effective. Documentation of the appearance of rat's back skin before sun exposure can be seen in Figure 3 and the appearance of rat's back skin after 21 days of exposure can be seen in Figure 4, where the erythema is marked by a red circle.

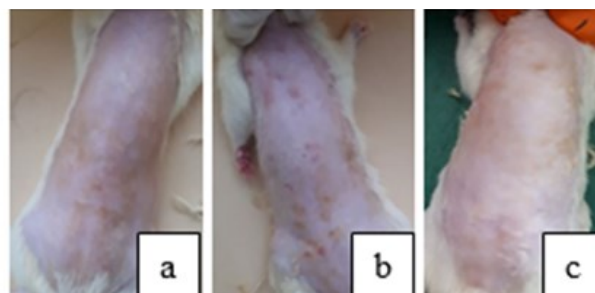


Figure 7. Rat's skin on the first day before sun exposure (H_0) (a) Group of negative control (b) Group of positive control (c) Treatment group [Kulit tikus sebelum dipapar matahari (H_0) (a) Kelompok kontrol negatif (b) Kelompok kontrol positif (c) Kelompok perlakuan].

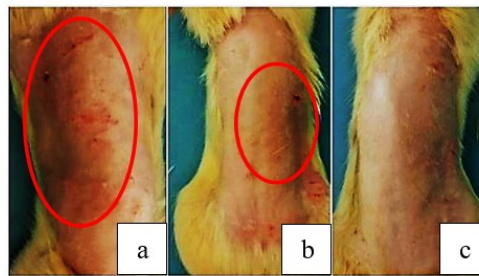


Figure 8. Rat's skin on the 21th day after sun exposure (H_{21}) (a) Group of negative control (1.242) (b) Group of positive control (0.792) (c) Treatment group (0.542) (*Kulit tikus setelah 21 hari pemaparan (H_{21}) (a) Kelompok kontrol negatif (1.242) (b) Kelompok kontrol positif (0.792) (c) Kelompok perlakuan (0.542)*)).

CONCLUSION

Based on the test results of *P. alba* leaf, it is shown that the leaves of *P. alba* have the ability to act as UV filters due to their flavonoid content. The measurement of flavonoid content in crude extract was 57.6 mg QE/g, while in pure extract was 64.4 mg QE/g. The results of pure extract testing at a concentration of 1400 mg/mL showed the best ability as a UV filter seen from the highest SPF value of 33.88. Application of pure extract with a concentration of 1400 mg/mL provides the best results in reducing erythema with a scoring result mean of 0.542, which indicates less erythema on the skin.

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