Jurnal Farmasi Galenika (*Galenika Journal of Pharmacy*) (e-Journal) 2025; 11 (1): 24 – 33
ISSN: 2442-8744 (electronic); 2442-7284 (printed)
Journal Homepage: https://bestjournal.untad.ac.id/index.php/Galenika
DOI: 10.22487/j24428744.2025.v11.i1.17347

In Vitro Evaluation of Sunscreen Activity of Extract and Fraction of Kedabu Fruit (*Sonneratia ovata* Backer)

(Uji Aktivitas Tabir Surya Ekstrak dan Fraksi Buah Kedabu (Sonneratia ovata Backer) Secara In Vitro)

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Article Info:
Received: 08 August 2024
in revised form: 07 October 2024
Accepted: 18 January 2025
Available Online: 1 March 2025

Keywords:
Kedabu Fruit
Sunscreen activity
SPF
%Te
%Tp

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ABSTRACT

The Kedabu fruit, scientifically known as *Sonneratia ovata* Backer, is a mangrove plant commonly found in Indonesia. This fruit is valued for its antioxidant properties and shows potential as a natural sunscreen. This study was conducted to determine the in-vitro activity of the extract and fractions of Kedabu fruit (*Sonneratia ovata* Backer) as a sunscreen, with the potential for development into a natural sunscreen preparation. Experiments were conducted on extracts and various fractions, including n-hexane, ethyl acetate, n-butanol, and water. The extracts and fractions of Kedabu fruit were prepared at concentrations of 200, 400, 600, 800, and 1000 µg/mL. Their absorbance was then measured in the UV A (320-375 nm) and UV B (290-320 nm) wavelength ranges. The n-butanol fraction, at a concentration of 1000 µg/mL, appears to be the most effective sunscreen, demonstrating a %Te value of 8.369% and %Tp of 50.345%. This performance places it in the standard Suntan range for UV B protection. Additionally, it has an SPF value of 10.58, categorizing it within the highest level of sun protection.

How to cite (APA 6th Style):
Lestari, P., Furi, M., Fernando, A., Amin, S., Irfan, N. (2025). In vitro evaluation of sunscreen activity of extract and fraction of kedabu fruit (*Sonneratia ovata Backer*). *Jurnal Farmasi Galenika: Galenika Journal of Pharmacy (e-Journal), 11*(1), 24 - 33. doi:10.22487/j24428744.2025.v11.i1.17347

INTRODUCTION

Mangroves have been known to provide ecological and socio-economic benefits, and they are growing very rapidly at this time. Mangrove trees are plant species that thrive in areas regularly affected by tidal changes (Pototan et al., 2021). Researchers studying mangroves have categorized these plants into true mangrove species and associated species. True mangroves are unique to mangrove environments, thriving exclusively in soggy conditions. In contrast, associated species can adapt to aquatic and terrestrial habitats and are often found in mangrove regions (Food Agriculture Organization, 2010).

The genus Sonneratia (family Lythraceae) is a group of mangroves exclusive to the Indomalaya region. It includes various species, including *S. caseolaris, S. alba, S. apetala, S. griffithii, S. hainanensis, S. lanceolata, and S. ovata* (Mao and Foong 2013). Mangrove forests are a type of forest located along the coast or river that is affected by the ebb and flow. Mangroves are plants that are rich in bioactive compounds and can be used as antioxidants. Mangroves have the potential as a source of highly bioactive compounds such as polyphenols, flavonoids and anthocyanins (Nengsih et al., 2021).

Chemical substances featuring one or more unpaired electrons, referred to as free radicals, are highly reactive due to their unstable nature. These free radicals seek to stabilize themselves by interacting with other molecules. Reactive oxygen species (ROS) are free radicals derived from oxygen, and can exist as ions, atoms, or molecules. While ROS are typically generated under normal conditions, excessive levels can be detrimental if not adequately removed, as they have the potential to trigger oxidative reactions in both living organisms and laboratory settings. Such reactions can lead to cell damage and oxidative stress, which may contribute to the development of various conditions including, aging, Parkinson's disease, Alzheimer's disease, cardiovascular disorders, and even cancer (Laili et al., 2018).

Excessive reactive oxygen species (ROS) can damage on DNA, leading to chromosome alterations and mutations. While cells possess various natural defense mechanisms against excess ROS, an imbalance in these systems may necessitate additional antioxidant sources to help combat the ROS. (Chanda & Dave, 2009). Herbal supplements derived from natural sources are a potent type of antioxidant. Traditionally, these supplements are recognized for their effectiveness in neutralizing free radicals (Laili et al., 2018).

Antioxidants are substances capable of donating their electrons to free radical molecules, which help to stabilize these radicals and prevent undesirable oxidation processes within cells (Wala et al., 2015). Utilizing compounds with antioxidant abilities can help prevent diseases linked to UV radiation exposure. Certain active antioxidant substances, including flavonoids, have been recognized for their protective qualities against UV rays (Hogade Maheshwar et al., 2010).

UV radiation exacerbates oxidative stress in skin cells, potentially triggering and advancing cancer development. There is growing interest in incorporating antioxidants into sunscreens to enhance their protective effects against UV exposure. Natural antioxidants offer promising new options for preventing and treating UV-related conditions. On Earth, the UV radiation that reaches the surface comprises roughly 40-99% UVA and 1-10% UVB (Rohman, 2016). Concerns have been raised about potential negative effects associated with synthetic sunscreen products. Limited research available on herbal sunscreens and their sun protection factor (SPF). Recently, natural substances have gained attention as potential sources of antioxidants for sunscreens, thanks to their ability to absorb UV light and their inherent antioxidant properties (Hashemi et al., 2019; Kale et al., 2010).

Flavonoids, classified as phenolic compounds, serve as effective photoprotectants because of their chromophore groups (conjugated double bonds), which can absorb ultraviolet radiation, including both UVA (320-400 nm) and UVB (290-320 nm). This absorption helps diminish the UV exposure on the skin (Walters et al., 1997). According to (Ebrahimzadeh et al., 2014), a strong relationship exists between the phenolic content in different plant extracts and their Sun Protection Factor (SPF) ratings.

Various compounds have been extracted from *S. ovata* Backer, and research has been carried out to examine their pharmacological effects. Nguyen et al. successfully isolated three new phenolic compounds, namely sonnerfenolic A, sonnerfenolic B, and sonnerfenolic C, and one cerebroside called sonnercerebroside. Sonnercerebroside extracted from Kedabu leaves demonstrated significant cytotoxic effects against MCF-7 cancer cells, achieving an IC_{50} value of 112.8±9.4 μM, and inhibiting acetylcholinesterase (AchE) activity. Wu et al. (2009), successfully isolated three compounds from Kedabu fruit, and the isolation outcomes indicated cytotoxic activity on rat glioma cell line C-6 with IC_{50} scores of 19.02; 20.21; and 31.77 μg/mL, respectively.

Research by (Basit, 2019) reported that the ethyl acetate fraction of kedabu leaves (*Sonneratia ovata* Backer) provides very strong antioxidant activity over DPPH with IC_{50} 12.47 μg/mL. (Putri, 2020) also noted that the n-butanol fraction of fruit (*Sonneratia ovata* Backer) provided very strong antioxidant activity over DPPH with IC_{50} 7.210 μg/mL, which was carried out by the ethyl acetate fraction with IC_{50} 17,433 μg/mL, ethanol extract with IC_{50} 75.34 μg/mL with strong category, water fraction with IC_{50} 76.098 μg/mL with strong category and *n-hexane* fraction with IC_{50} 141.19 μg/mL with moderate category.

Based on several previous studies, kedabu fruit has the potential as a sunscreen. Therefore, further studies are needed on the sunscreen activity test of ethanol extract and fractions of kedabu fruit (*Sonneratia ovata* Backer). The sunscreen activity test was carried out in vitro by deciding the percentage score of erythema Transmission (%Te), percentage of pigmentation Transmission (%Tp),

and Sun Protection Factor (SPF) score. This study is expected to provide an overview and source of information on the sunscreen activity skills of kedabu fruit extract and fractions, as well as a source of natural ingredients that can be developed into sunscreen cosmetic products.

MATERIAL AND METHODS

Tools and Materials

The equipment used included an analytical balance, glass tools laboratory, rotary evaporator (Buchi® 461 Water Bath), *96 well microplates* (Costar 3596®), *96 microplate reader* (Epoch BioTek®), and multichannel micropipettes (Nexty®). This study utilized various materials, including ethanol extract from Kedabu fruit (*Sonneratia ovata Backer*), ethanol (Merck®), n-hexane (Merck®), ethyl acetate (Merck®), n-butanol (Merck®), and distilled water.

Methods

Fractionation

Thirteen grams of ethanol extract from kedabu fruit were weighed and mixed with 50 mL of distilled water. The mixture was stirred the extract was completely dissolved. This mixture was then transferred to a separating funnel, and 50 mL of n-hexane was added. The funnel was shaken for a few minutes and allowed to sit until two distinct layers formed. The lower water layer was carefully drained through the tap of the separating funnel, which was repeated three times.

The water layer, fractionated with n-hexane, was then fractionated with 50 mL of ethyl acetate. The water layer was removed through the separating funnel tap. The next step was fractionation using n-butanol. Later, 50 mL of n-butanol were added to water, and the mixture was shaken and left to stand until two layers were formed. The water and n-butanol layers were then separated. Finally, the fractionation outcomes were concentrated using a rotary evaporator.

Preparation of Test Solution

10 mg of ethanol extract and kedabu fruit fractions were taken and dissolved in absolute ethanol. The solution was taken transferred to a 10 mL volumetric flask, add absolute ethanol was added until the solution reached the limit mark. The solution was homogenized until a test solution with a concentration of 1000 µg/mL was achieved. Solutions of 100 µL, 80 µL, 60 µL, 40 µL, and 20 µL were taken, respectively, then placed into wells A, B, C, D, and E, while well F was filled with a blank solution of absolute ethanol in the amount of 100 µL that had been labeled. Wells B, C, D, and E were supplemented with absolute ethanol to volume of 100 µL, resulting in test solutions with varying concentrations of 1000 µg/mL, 800 µg/mL, 600 µg/mL, 400 µg/mL, and 200 µg/mL.

Measurement of sunscreen activity

The solutions with concentrations of 1000 µg/mL, 800 µg/mL, 600 µg/mL, 400 µg/mL, and 200 µg/mL, along with a blank solution, were assessed for absorbance using a microplate reader. Measurements were taken within the wavelength ranges of 293-318 nm for %Te and 323-373 nm for %Tp, with intervals of 5 nm. Afterwards, the outcomes were entered into the equation: (Balsam, M & Sagarin, 1972)

$$\% \text{ Te} = \frac{Ee}{\sum Fe} = \frac{\sum(T \; x \; Fe)}{\sum Fe}$$

$$\% \text{ Tp} = \frac{Ep}{\sum Fp} = \frac{\sum(T \; x \; Fp)}{\sum Fp}$$

Description:

T: Transmission

Fe: The intensity of erythema at a given wavelength

Tp: The proportion of erythema intensity that passes through the sunscreen

Fp: The intensity of pigmentation at a specific wavelength

Ep: The proportion of pigmentation intensity that is transmitted through the sunscreen

To determine the SPF score, the test solution and blank were measured using a microplate reader at a wavelength range of 290-320 nm with an interval of 5 nm. The measurement outcomes were then entered into the equation (Mansur et al., 1986)

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

Description:

CF: Scored correction factor 10

EE: Erythemogenic effect of radiation at wavelength (λ)

I: Sunlight simulation spectrum intensity (λ)

A: Absorbance at wavelength (λ)

RESULT AND DISCUSSION

The yield of the different fractions was calculated using 13 grams of the separated ethanol extract. The results showed that the water fraction was 70.09%, the n-hexane fraction was 0.25%, the ethyl acetate fraction was 7.73%, and the n-butanol fraction was 5.38%. In order to identify groups of secondary metabolite chemicals present in the kedabu fruit extracts and fractions, this screening was done as a preliminary test. The results of the phytochemical research showed that the ethanol extract of kedabu fruit included phenolic, flavonoid, terpenoid, and steroid components. The n-hexane fraction contained terpenoid and steroid chemicals, while the ethyl acetate fraction contained phenolic, flavonoid, terpenoid, and steroid chemicals. Phenolic and flavonoid molecules were also detected in the water and n-butanol fraction. Phytochemical screening is a crucial step in determining whether medicinal plants have the potential to act as antibiotics, antioxidants, or anticancer agents(Astuti et al., 2013).

The erythema/pigmentation transmission percentage is the ratio of the amount of UV light energy transmitted by the sunscreen preparation in the erythema/pigmentation spectrum to the number of erythema effectiveness factors at each wavelength in the range of 292.5–337.5 nm (Eff et al., 2018). The %Te and %Tp scores of the kedabu fruit extracts and fractions were computed in order to test the sunscreen activity. The percentage score, which measures the amount of UV light energy absorbed by UV B radiation (290–320 nm), was developed to characterize a chemical compound's capacity to shield the skin from UV rays that induce erythema. Within two to three hours of being exposed to sunlight, erythema, a condition in which the skin becomes red due to an inflammatory reaction, manifests The %Tp score, which measures the quantity of UV light energy absorbed by UVA radiation (320–375 nm), was computed to characterize a chemical compound's capacity to shield the skin from UV rays that result in pigmentation. The darkening of skin tone brought on by exposure to UV rays is known as pigmentation (Hasanah et al., 2015).

Table 1. Sunscreen Category Based on %Te and %Tp scores of Extracts and Fractions of Kedabu Fruit (*Sonneratia ovata* Backer).

Concentration (µg/mL)	**Ethanol Extract**		**Category**
	%Te	**%Tp**	
1000	35.343	55.648	(-)
800	39.673	62.623	(-)
600	48.995	70.598	(-)
400	64.752	81.693	(-)
200	82.496	91.443	(-)
Concentration (µg/mL)	**n-hexane fraction**		**Category**
	%Te	**%Tp**	
1000	52.804	65.678	(-)
800	62.643	72.697	(-)
600	67.267	76.697	(-)
400	78.398	83.751	(-)
200	88.228	89.752	(-)
Concentration (µg/mL)	**Ethyl acetate fraction**		**Category**
	%Te	**%Tp**	
1000	14.05	61.752	*Fast Tanning*
800	19.743	67.786	(-)
600	27.919	74.757	(-)
400	41.134	83.051	(-)
200	60.38	91.848	(-)
Concentration (µg/mL)	**n-butanol fraction**		**Category**
	%Te	**%Tp**	
1000	8.369	50.345	*Standard Suntan*
800	12.831	58.321	*Fast Tanning*
600	19.689	66.933	(-)
400	33.314	79.019	(-)
200	51.312	87.587	(-)
	Water Fraction		**Category**

Concentration (µg/mL)	%Te	%Tp	
1000	68.269	90.753	(-)
800	74.127	95.171	(-)
600	75.966	96.337	(-)
400	78.542	97.267	(-)
200	81.399	99.253	(-)

Description: (-) = Not categorized as sunscreen

Based on the grouping of sunscreen categories according to %Te and %Tp scores, the ethyl acetate fraction with a %Te score of 14.05% and %Tp of 61.752% is included in the fast-tanning category at a concentration of 1000 µg/mL. Meanwhile, the n-butanol fraction at a concentration of 1000 µg/mL has a %Te score of 8.369% and %Tp of 50.345%, which is categorized as a standard suntan. At a concentration of 800 µg/mL, the n-butanol fraction indicates a %Te score of 12.831% and %Tp of 58.321%, which is also included in the fast-tanning category.

Standard suntan refers to a classification of sun protection where a material has the ability to absorb more than 95% of UVB radiation, while avoiding pigmentation without causing erythema. The standard sun protection category is effective in preventing erythema on healthy skin. The rapid injection stage is the ability of a chemical in a sunscreen to instantly darken the skin without causing erythema by allowing UVA radiation to penetrate completely to create an optimal darkening effect (Whenny et al., 2015). Furthermore, sunscreen activity testing is carried out based on the FPM score by evaluating the absorbance of the test sample in the wavelength range of 290-320 nm.

Table 2. Sun Protector Factor (SPF) score of n-butanol fraction

n-butanol fraction		
Concentration (µg/mL)	**SPF**	**Category**
1000	10.58	Maximal Protection
800	8.06	Maximal Protection
600	6.11	Extra Protection
400	3.78	Minimal Protection
200	1.75	(-)

The sun protection factor (SPF) was used to represents the ability of a sunscreen to delay the onset of sun-induced skin erythema, which is a sign of damage caused by UV B radiation (Baumann, 2009). According to the Table. 1, the study found that the n-butanol fraction exhibited best sunscreen activity based on the %Te, %Tp score graph and the n-butanol fraction SPF score graph. As the concentration of the test sample increased, the %Te and %Tp scores decreased, while the SPF score increased.

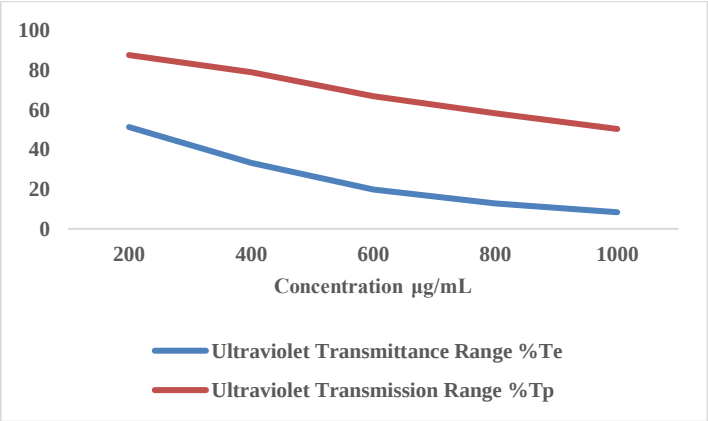

Figure 1. %Te and %Tp score graph of n-butanol fraction of Kedabu fruit (*Sonneratia ovata* Backer).

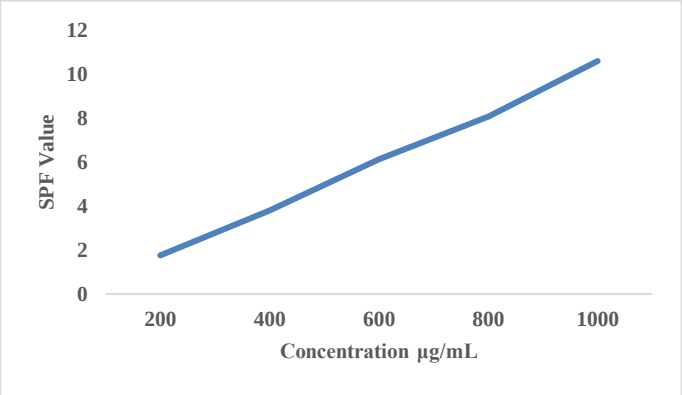

Figure 2. SPF score graph of n-butanol fraction of Kedabu fruit (*Sonneratia ovata* Backer).

The findings of this research are further validated by phytochemical tests that show the presence of phenolic and flavonoid compounds in the n-butanol fraction. The higher the total phenol and flavonoid values, the higher the antioxidant'sability to suppress the free radicals's development. Additionally, (Furi et al., 2020) observed that the n-butanol fraction had the highest levels of total phenolics and flavonoids compared to ethanol extracts and other fractions of the Kedabu fruit. These phenolic and flavonoid compounds contain chromophore groups (conjugated double bonds) that can absorb UV radiation, making them crucial for sunscreen activity.

At a concentration of 1000 µg/mL, the n-butanol fraction of *Sonneratia ovata* Backer fruit proved to be the most effective sunscreen. It achieved the requirements for efficient UV B protection at this concentration with a %Te score of 8.369% and a %Tp score of 50.345%. Furthermore, the SPF score of 10.58 shows maximal protection, implying that the skin's natural resistance to redness and darkening caused by sun exposure was increased by 10.58 times.

CONCLUSION

The study concludes that the n-butanol fraction of Kedabu fruit (*Sonneratia ovata* Backer) at a concentration of 1000 μg/mL exhibits the highest potential as a sunscreen. At this concentration, the %Te score reached 8.369%, and the %Tp was 50.345%, classifying it under effective sun protection standards against UVB rays. Furthermore, it achieved an SPF value of 10.58, categorizing it in the maximum protection level.

CONFLICT OF INTEREST

The author states that there are no conflicts of interest related to the preparation of this article.

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