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Sunscreen Activity Test of Tahongai (*Kleinhovia hospita* L) Leaves Infusion use UV-Vis Spectrophotometry Method

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ABSTRACT

Excessive frequency and duration of UV exposure causes adverse effects, including erythema, pigmentation, premature aging, and even skin cancer. Therefore, additional protection is required, such as the use of sunscreen. The development of natural active ingredients is necessary to reduce the negative impact of sunscreen products. Tahongai (*Kleinhovia hospita* L) contains flavonoid compounds that have the potential as sunscreens due to the presence of chromophore groups that can absorb UVA and UVB rays. The purpose of this study was to determine the potential of tahongai leaf infusion as a sunscreen. The test method of sunscreen activity was carried out in-vitro using UV-Vis spectrophotometry. The sunscreen activity test was carried out by calculating the percent transmission value (%Te), the percent transmission value of pigmentation (%Tp), and the SPF value. The results of the calculation of %Te, %Tp, and SPF values, as well as the sunscreen category at a concentration of 0.5 g are %Te of 3.368 (extra protection), %Tp of 1.248 (sunblock), and SPF value of 21.945 (ultra protection). Tahongai leaf infusion has potential as an active ingredient for sunscreen products and still requires further in vivo research to ensure its effectiveness as well as the duration of its protection.

Keywords: *Kleinhovia hospita* L, SPF, Sunscreen

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INTRODUCTION

Excessive exposure to ultraviolet (UV) radiation from sunlight has the potential to cause erythema (reddening) due to UV radiation at wavelengths of 290-320 nm (UV-B) and skin darkening at wavelengths of 320-400 nm (UV-A) (Moore el al, 2013). Erythema is followed by melanocyte activation which increases the rate of melanin production (increases melanization), while the long-term effects can lead to irreversible loss of skin elasticity and can lead to the development of skin cancer, both melanoma and nonmelanoma (Harrison and Bergfeld, 2009). The extent of skin damage depends on the frequency and duration of exposure, if excessive exposure causes the natural protection system to be unable to withstand radiation, so additional protection is needed, including the use of sunscreen (Lintner, 2017).

Sunscreens are substances that can effectively scatter, reflect, or absorb sunlight. Nowadays, more and more evidence show that the use of sunscreens is not completely safe due to synthetic ingredients that have toxic and irritating effects on the skin (Ardiansyahd dkk, 2022). The development of natural materials is an important source for research in the discovery of new active compounds. It offers the discovery of new biological mechanisms.

Tahongai (*Kleinhovia hospita* L) belongs to the Malvaceae family and is widely grown in East Kalimantan. Tahongai has several pharmacological activities, including as an antidiabetic, antioxidant, and anticancer (Arung et al, 2012 ; Rahim dkk, 2018), and hepatoprotector (Zhou et al, 2013). Known active compounds possessed by tahongai, including scopoletin, quartetin, rutin, and chamferol. Based on the results of research conducted by Arung et al. (2012) methanol extract of tahongai leaves showed strong antioxidant activity (96%), when compared to vitamin C (98%) using the DPPH method. The active compounds eleutherol and kaempferol 3-O-B-D-glucoside of tahongai leaves have antioxidant activity with DPPH examination (IC50 kaempferol 71.4 µM and eleutherol 491.8 µM) (Arung et al, 2012).

Quantitatively, the ethanol extract of tahongai leaves contains 2.83% alkaloids, 19.78% flavonoids and 14.23% saponins and contains flavonoids indicated by the presence of O-H bonds, C=C double bonds and C-H bonds (Aji dkk, 2021). Flavonoid compounds contained in tahongai leaves have potential as sunscreens. Flavonoid compounds contain chromophore groups which are conjugated aromatic groups that cause the ability to absorb strong light in the UV wavelength range (Yuni dkk, 2021). Tahongai leaves may have a potential sunscreen profile as an active compound in cosmetic products. Therefore, this study was conducted to determine the potential of tahongai leaf infusion as a sunscreen.

MATERIALS AND METHODS

Tools and Materials

Tools used in this study, UV-Vis Spectrophotometer, oven (Memmert UN55), centrifugation (Zenithlab LC-04S), hot plate (SP 131320-33Q), analytical balance (Fujitsu FS-AR), waterbath, a thermometer, stopwatch, and laboratory glassware.

Jurnal Farmasi Galenika Vol. 13 No. 1
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Materials used in this study, Tahongai leaves (*Kleinhovia hospita* L), ethanol pro analysis (Merck), n-hexane, iron (III) chloride, Mg metal, concentrated sulfuric acid, hydrochloric acid, mayer reagent, dragendorff, bouchardat, distilled water, and filter paper, and aluminium foil.

Raw Material Collection

The sample to be studied is tahongai leaves obtained from a community plantation in Lempake Village, North Samarinda.

Plant Determination

Plant determination is intended to determine the truth of the sample. Determination was carried out at "Herbarium Mulawarman", Laboratory of Ecology and Biodiversity Conservation of Tropical Forests, Faculty of Forestry, Mulawarman University, Samarinda.

Sample Preparation

The tahongai leaves obtained were wet sorted to separate from dirt and stems. Selected dark green leaves and no holes, then washed with running water and dried in an oven at 60 °C until simplicia is obtained which when squeezed rustles and turns into dry leaf flakes (Aji dkk, 2021).

Preparation of tahongai leaf infusion

Infundation of 50 g of dried tahongai leaves was carried out with 50 mL of water solvent at 90 °C for 15 minutes. Heating was carried out on a hot plate for 15 minutes starting from the temperature reaching 90 °C while repeatedly stirring. The variant was filtered while still hot using filter paper (Aji dkk, 2021).

Phytochemical screening of tahongai leaf simplicia and infusion

Alkaloids

A sample of 0.5 g was added with 1 mL of 2 N hydrochloric acid and 9 mL of distilled water, heated on a waterbath for 2 minutes, cooled, and filtered. Prepared 3 test tubes, each tube inserted 0.5 mL filtrate. Each test tube was added 2 drops of mayer, bouchardat, and dragendorff reagents. Positive results contain alkaloid compounds if a precipitate occurs. If 2 of the 3 reagents above are positive for alkaloid compounds, the sample is declared to contain alkaloids, namely the formation of a white or yellow precipitate (Handayani dkk, 2020).

Flavonoids

A sample of 1 g was added to 10 mL of distilled water, then boiled for 5 minutes, filtered while still hot. The filtrate obtained was taken as much as 5 mL, added 0.1 magnesium powder, 1 mL HCl and 2 mL amyl alcohol, shaken and waited until it separated. Positive results contain flavonoid compounds if there is a yellow red color change in the filtrate or red orange color in the amyl alcohol layer (Aji dkk, 2021).

Saponins

A sample of 0.5 g was put into a test tube, added 10 mL of distilled water, cooled, then shaken vigorously for 15

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minutes. If foam forms as high as 1-10 cm and when dripped with 2 N hydrochloric acid the foam still exists, then the sample contains saponins (Aji dkk, 2021).

Tannins

A sample of 1 g in 10 mL of distilled water is brought to a boil, then cooled and filtered. The filtrate obtained is diluted with distilled water until clear or colorless. Taken 2 mL of solution, added with 1-2 drops of 5% iron (III) chloride reagent. Observed the color change that occurs, if the color turns blue or blackish green, then the sample contains tannins (Taupik dkk, 2022).

Phenolic

A sample of 1 g sample is added with FeCl3 solution. The formation of a green or blackish green color indicates positive phenol (Taupik dkk, 2022).

Terpenoids and steroids

A sample of 0.5 g was macerated with 10 mL of n-hexane for 1 hour and then filtered using filter paper. The filtrate obtained was evaporated, the remaining filtrate was added with 10 drops of anhydrous acetic acid reagent and 1 drop of concentrated sulfuric acid. Observed changes that occur, if the positive sample contains terpenoid or steroid compounds, it will be marked by the formation of purple or red color that turns blue green (Taupik dkk, 2022).

Determination of SPF Value, %Te Value, and %Tp Value

A total of 0.5 g of tahongai leaf infusion was dissolved in 25 mL of ethanol p.a. Shaken and centrifuged until the filtrate was obtained. Then the absorbance was measured using UV-Vis Spectrophotometer with wavelength of 290-320 nm (SPF), wavelength of 292.2-372.5 nm (%Tp), and wavelength of 290-400 (%Te) nm with interval of 5 nm and ethanol p.a as blank. The test was conducted three times (Hasanah dkk, 2015).

Data Analysis

Data analysis was done descriptively in the form of calculations of %Te value, %Tp value, and SPF value. Conducting testing three times.

Calculation of % Te value and % Tp value

% Te and % Tp are calculated based on the formulas below:

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

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

Description:

Te : Percentage value of erythema transmission

Fe : Erythema flux whose value is at a wavelength of 292-317 nm

Ee : The amount of erythema flux that is transmitted by the sunscreen

Tp : Pigmentation transmission percentage value

Ep : Amount of pigmentation flux transmitted by the sunscreen

Calculation of the SPF value

The calculation of SPF value follows the equation of Mansur (1986). The equation is as follows:

$$SPF = CF \; x \Sigma_{290}^{320} \; EE \; (\lambda) \; x \; A \; (\lambda)$$

Description:
CF : Correction factor worth 10
EE : Erythmogenic effect of radiation at wavelength (λ)
I : Simulated spectrum of sunlight (λ)
A : Absorbance value at wavelength (λ)

RESULT AND DISCUSSION

Phytochemical Screening of Simplicia and Infusion of Tahongai Leaves

Phytochemical screening in this study aims to determine the class of compounds contained in tahongai leaves. Phytochemical screening is one of the simple, fast, and selective methods that can be used to identify groups of compounds and the presence of active compounds contained in tahongai leaves. The screening method is done by looking at the color test reaction using a color reagent. In this study, the results of phytochemical screening of simplicia and infusion of tahongai leaves are presented in Table 1.

Table 1. Phytochemical screening of simplicia and infusion of tahongai leaves

Screening	Simplicia	Infusion
Alkaloid		
Dragendroff	+	-
Mayer + HCl	+	-
Bouchardat	+	+
Flavonoids	+	+
Phenolic	-	+
Saponins	+	+
Tannins	+	+
Terpenoids and steroids	+	-

Description
\+ : Positive
\- : Negative

The method used in this study is infundation. Infundation is used to extract the active content of water-soluble simplicia. This method is very simple and the time required is relatively short. Samples that have been infundated are immediately carried out phytochemical screening tests and tested for sunscreen activity.

Determination of %Te, %Tp, and SPF value

Based on the results of research that has been carried out using a UV-Vis spectrophotometer in-vitro, the test results are presented in Table 2, Table 3, Table 4, and Table 5.

Table 2. %Te test results of tahongai leaf infusion

	λ (nm)	Transmittance			T	Fe	T x Fe	%Te
		1	2	3				
%Te	292,5	2,642	2,66	2,678	2,66	0,1105	0,29393	3,368864
	297,5	2,499	2,506	2,525	3,765	0,672	2,53008	
	302,5	2,257	2,28	2,276	3,4065	1	3,40065	
	307,5	2,02	2,065	2,038	3,0615	0,2008	0,614749	
	312,5	1,843	1,901	1,848	2,7796	0,1364	0,381374	
	317,5	1,715	1,784	1,716	2,6075	0,1125	0,293344	
						2,2322	7,519977	

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Erythema transmission is the amount of UV light energy that is transmitted at UV wavelengths of 290-320 nm (UV-B). The percentage value of erythema transmission (%Te) is a value that describes the ability of a chemical compound to protect the skin from UV-B rays that can cause erythema (redness) (Hasanah dkk, 2015). Based on Table 2. Tahongai leaf infusion has a %Te of 3.368 which is included in the extra protection category because it is in the range (1-6). Extra protection in the sense of sunscreen is the ability of a chemical molecule to protect sensitive skin by absorbing 95% or more of UV radiation at a wavelength of 290-320 nm, thus protecting more skin from exposure to UV-B rays that cause painless skin erythema and prevent skin darkening (Dutra et al, 2004).

Table 3. %Tp test results of tahongai leaf infusion

	λ (nm)	Transmittance			T	Fp	T x Fp	%Tp
		1	2	3				
%Tp	322,5	1,672	1,706	1,629	1,669	0,1079	0,18008	1,24818
	327,5	1,546	1,63	1,547	1,57433	0,102	0,16058	
	332,5	1,455	1,537	1,452	1,48133	0,0936	0,13865	
	337,5	1,354	1,444	1,354	1,384	0,0798	0,11044	
	342,5	1,059	1,126	1,063	1,08266	0,0669	0,07243	
	347,5	0,991	1,055	0,991	1,01233	0,057	0,05770	
	352,5	0,997	0,982	0,907	0,962	0,0488	0,04694	
	357,5	0,805	0,883	0,806	0,83133	0,0456	0,03790	
	362,5	0,711	0,796	0,713	0,74	0,0356	0,02634	
	357,5	0,623	0,709	0,624	0,652	0,031	0,02021	
	372,5	0,553	0,642	0,557	0,584	0,026	0,01518	
						0,6942	0,86649	

Pigmentation transmission is the amount of UV light energy that is transmitted at wavelengths of 320-375 nm (UV-A), which is the difference with the measurement of erythema transmission. The percentage value of pigmentation transmission (%Tp) is the ability of a compound to protect the skin from UV-A rays. Based on the tests that have been carried out, the %Tp is 1.248 (Table 3) which is included in the sunblock category. Sunblock in the sense of sunscreen, which is the ability of a chemical molecule to totally protect sunlight against skin that is very sensitive to UV-A rays at a wavelength of 322.5-372.5 nm and UV-B 292.5-337.5 nm which causes erythema and pigmentation from ultraviolet light (Dutra et al, 2004).

Table 4. SPF value of tahongai leaf infusion

	λ (nm)	Abs			A	EE x I	Abs x EE x I	CF	SPF
		1	2	3					
SPF	290	2,6155	2,6588	2,6667	2,64666	0,015	0,0397	10	21,9455
	295	2,5778	2,6099	2,6266	2,60433	0,0817	0,2127743		
	300	2,3888	2,3998	2,4048	2,398	0,2874	0,6891852		
	305	2,114	2,1766	2,1577	2,15766	0,3278	0,7072831 3		
	310	1,9377	1,9888	1,9422	1,95566	0,1864	0,3645362 6		
	315	1,7772	1,8366	1,7775	1,79433	0,0839	0,1505445 6		
	320	1,667	1,7466	1,6672	1,696	0,018	0,030528		
	Totals						2,1945512		

Jurnal Farmasi Galenika Vol. 13 No. 1
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Table 5. Sunscreen protection categories based on %Te, %Tp, and SPF

Determination	Result	Sunscreen profile
SPF	21,945	Ultra protection
%Te	3,368	Extra protection
%Tp	1,248	*Sunblock*

SPF describes the ability of a sunscreen to protect the skin from erythema. SPF is the ratio of the duration of UV radiation required to produce erythema on protected skin (with sunscreen) that is required to produce erythema on unprotected skin (without sunscreen). Therefore, the SPF value can tell the UV-B protection efficacy of a sunscreen product (Novia dkk, 2020). The higher the SPF value of a sunscreen active substance, the more effective it is to protect the skin from the adverse effects of UV rays. A chromophore group is a part of a molecule that is capable of absorbing UV radiation because it has a conjugated double bond system such as C=C and C=O. Electron delocalization in this system allows electron excitation when exposed to UV light. The longer the conjugated system, the greater the wavelength absorbed, thereby increasing the compound's ability to absorb UV radiation. (Purwaningsih dkk, 2015). Flavonoids are phenolic compounds with a C6–C3–C6 structure that have a strong chromophore system and hydroxyl groups as auxochromes. This structure enables flavonoids to effectively absorb UV light. The longer the conjugated system and the more –OH groups, the higher the UV absorbance capacity and SPF value. A sunscreen is said to provide protection if the SPF value is at least 2 and a good category if the SPF value is above 15 which is included in the ultra-protection category. Based on the test results obtained, tahongai leaf infusion has an SPF value of 21.945 which is categorized as providing ultra protection which means it can inhibit ultraviolet radiation by 96.0-97.4%. Ultra protection means being able to provide better protection from the risk of long-term skin damage, such as skin cancer. In addition, SPF above 15 is able to protect the skin longer from sun exposure (Aji dkk, 2021). The sunscreen potential possessed by tahongai leaf infusion is influenced by the presence of flavonoid compounds that act as strong antioxidants and metal ion binders believed to be able to prevent the harmful effects of ultraviolet rays or can reduce skin damage. The infusion of tahongai leaves tested positive for phenol, which is a natural plant antioxidant compound. The protective effect of phenol comes from the capacity to transfer free radical electrons, catalyze metal chelates, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidation. Phenols protect against solar UV radiation and reflect UV generated

ROS (Reactive Oxygen Species) (Novia dkk, 2020).

CONCLUSION

Tahongai leaf infusion has potential as a sunscreen with a sunscreen profile %Te of 3.368 (extra protection), %Tp of 1.248 (sunblock), and SPF value of 21.945 (ultra protection), and still requires further in vivo research to ensure its effectiveness as well as the duration of its protection.

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ADDITIONAL INFORMATION

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