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Research Article

Determination of SPF Value and Total Tannin in Ethyl Acetate Fraction of *Peronema canescens* Jack

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ABSTRAK

Daun Sungkai (Peronema canescens Jack) mengandung metabolit sekunder antara lain tannin, alkaloid, terpenoid, steroid, dan flavonoid. P. canescens merupakan tanaman asli Kalimantan Selatan yang berpotensi sebagai perawatan kulit alami berdasarkarkan kandungan tannin yang memiliki aktivitas antioksidan sehingga dapat dikembangkan sebagai produk tabir surya. Penelitian ini bertujuan untuk menentukan nilai SPF dan tannin total fraksi etil asetat daun Sungkai (P. canescens) menggunakan spektrofotometri UV-Vis. Metode ekstraksi dilakukan dengan maserasi menggunakan etanol 96% dan penggunaan fraksi n-heksana dan etil asetat. Penentuan tanin total menggunakan standar katekin dengan konsentrasi 40,60, 80, 100 dan 120 ppm. Hasil kadar tannin total sebesar 0,5586% ±0.01516 ekivalen katekin dan nilai SPF sebesar 5.703±0.021. Dengan demikian dapat disimpulkan daun Sungkai (Peronema canescens Jack) dapat dikembangkan sebagai tabir surya alami.

Kata Kunci: Daun Sungkai, Tabir Surya, Tannin Total, UV-Vis, SPF

ABSTRACT

Sungkai leaves (*Peronema canescens* Jack). contains secondary metabolites i.e tannins, alkaloids, terpenoids, steroids, and flavonoids. *P. canescens*, natural plant from South Kalimantan, has potential benefits as natural skincare based on tannin which contains antioxidant activity that can be developed as a sunscreen product. This study aimed to determine the sun protection factor (SPF) and total tannin ethyl acetate fraction of sungkai leaves (*P. canescens*) using UV-vis spectrophotometry. The extraction method was maceration with 96% ethanol and the fraction used n-hexane and ethyl acetate. The determination of total tannin with catechin standards concentration 40,60,80, 100 and 120 ppm. The result of total tannin was 0.5586% ±0.01516 catechin equivalents and the SPF value was 5.703±0.021. The conclusion of the research is that *Peronema canescens* Jack can be developed into natural sunscreen.

Keywords: Sungkai Leaves, Sunscreen, Total Tannin, UV-Vis, SPF

I. INTRODUCTION

Indonesia is a tropical country where exposure to sunlight is greater than in sub-tropical countries. Excessive sun exposure due to UV radiation on the skin can cause some damage, including redness, itching, and burning skin, causing premature aging erythema to skin cancer (Widyawati *et al.,* 2019). To avoid these effects, protection is needed on the skin, especially topicals that can reduce the dangers of ultraviolet rays, one of which is sunscreen (Fransisca, 2020). Sunscreen is a preparation that can reduce skin damage due to direct exposure to UV rays by absorbing these UV rays (Zarkogianni & Nikolaidis, 2016).

The ability of an effective sunscreen can be seen by determining the value of the Sun Protection Factor (SPF), which is an indicator of a UV protective substance where the higher the SPF value, the higher the protection given to the skin, thus reducing the occurrence of sunburn on the skin (Paliyath *et al.,* 2011). The use of commercial sunscreens currently still uses a lot of chemicals and these materials can cause allergic reactions so the use of natural sunscreens is introduced by the public. One of the natural ingredient is Sungkai Plant (*Peronema canescens* Jack).

Sungkai Leaves (*P. canescens* Jack) contain secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds. One of the secondary metabolites that as an antioxidant is a tannin which is a polyphenol compound with a high molecular weight of around 500 to 3000 Da (Dalton) and is a typical Kalimantan plant that is widely used as a medicine for malaria, fever, mouthwash and other properties (Fatonah *et al.,* 2021). Tannins are known to act as biological antioxidants (Hidayah, 2016), so they can stabilize free radicals which can protect the skin from sun exposure (Malangngi, Sangi, Paendong, 2017). The antioxidant activity of tannins is stronger as polyphenolic compounds than simple phenols (Yulianti *et al.,* 2015). Research conducted by Fadlilaturrahmah, Sungkai leaf plants has antioxidant activity with an IC_{50} value of 44.933 ppm so they are included in very active antioxidants. Determination of the SPF value of the ethanol extract of *P. canescens* with concentrations of 600 ppm 24 ± 0.31, 400 ppm 16 ± 0.34 and 200 ppm 8 ± 0.3 (Fadlilaturrahman, 2021). In determining the antioxidant activity of the ethanol extract of young and old leaves of *P. canescens*, IC_{50} values were obtained of 50.83 and 52.83 ppm respectively

(Okfrianti & Irnameria, 2022). The content of *P. canescens* which act as antioxidants has the potential to be used as a natural sunscreen, so it is necessary to determine the SPF value. Several studies have been conducted using *P. canescens* leaf extract, however, fractions have not been prepared. Fractination is carried out to determine secondary metabolites based polarity. Determination of fraction *P. canescens* was due to knowing more specific secondary metabolit, and to complete the data for next experiment.

Thus, in this study the total tannin content and effectiveness of the ethyl acetate fraction of *P. canescens* sunscreen were determined using UV-Vis spectrophotometry.

II. METHODS

A. Materials

The tools used were 25 mesh sieve, stirring rod, evaporator cup, glass funnel, beaker glass, measuring cup, hotplate (Stirrer Thermo), measuring flask, macerator, analytical balance (SI 6002), dropping pipette, volume pipette, propipet, rack tubes, test tubes and UV-Vis spectrophotometry (*Spectronic Genesys® 10 UV*). The materials used were P. canescens leaves, catechins (*Sigma Aldrich®),* distilled water, hydrochloric acid, 96% ethanol (*technical®*). ethanol p.a, ethyl acetate (*technical®*), n-hexane (*technical®*), and 1% vanillin.

B. Preparation of *P. canescens* leaf ethanol extract

Sungkai leaves Simplicia as much as 700 grams, macerated with 96% ethanol in a maceration vessel. 96% ethanol was added until all the leaf powder was evenly wetted. The sample is stirred every 6 hours and filtered from the solvent every 24 hours using filter paper and then a new solvent is added. Extraction was carried out for 3×24 hours. The filtrate is evaporated with a waterbath until a thick extract with a constant weight is obtained (Peni Pindan *et al.,* 2021)

C. Preparation of Ethyl Acetate Fraction from *P. canescens* leaves

The ethanol extract of *P. canescens* leaves as much as 20 grams and then suspended in distilled water and ethyl acetate with a ratio (1:2) and stirred until homogeneous. The remaining water layer was then fractionated using 40 mL ethyl acetate. Ethyl acetate fractions were separated in different containers.

D. Catechin Standard Curves

Assay series were prepared using catechins as a standard. A total of 10.0 mg of catechins was dissolved in 10.0 mL of ethanol p.a. 1000 ppm standard solution

was obtained. Making a series of catechin levels is done by taking as much as 0.4; 0.6; 0.8; 1.0 and 1.2 mL of a catechin solution with a concentration of 1000 ppm, then each was put into a 10 mL volumetric flask and added with ethanol p.a up to the mark. Catechin solutions were obtained at concentrations of 40, 60, 80, 100, and 120 ppm. As much as 0.5 mL of the serial concentration solution was taken, added with 3.0 mL of 1% vanillin solution, and vortexed. 1.50 ml of concentrated HCl was added, vortexed, and allowed an operating time of 16 minutes references used 15-20 minutes, (Fadlilaturrahmah, 2021) at room temperature. Read the absorbance series of levels using a UV-Vis spectrophotometer at a maximum wavelength of 501 nm, so that concentrations are obtained and standard curves for catechins are made. These results are made into a standard curve equation y = bx + a. The blank used consisted of all the reagents used with the addition of ethanol p.a.

E. Determination of Total Tannin Ethyl Acetate Fraction

Determination of the tannin content of the ethyl acetate fraction can be carried out by weighing 500.0 mg of the ethyl acetate fraction, then putting it into a 10.0 mL flask, and dissolving it with solvent up to the mark. Take 0.5 mL of the mixed solution, add 3.0 mL of 1% vanillin solution, and vortex. As 1.5 mL of concentrated HCl was added to the solution, vortexed, and incubated for 16 minutes at room temperature. Absorbance readings were carried out with a UV-Vis spectrophotometer at a maximum wavelength of 501 nm (Pratama *et al.*, 2019).

F. SPF Value of the Ethyl Acetate Fraction of *P. canescens* Leaves

The UV-Vis spectrophotometer is calibrated first using 1 mL of distilled water into the cuvette Then the fraction was weighed, dissolved with ethyl acetate, and shaken with a vortex for 5 minutes to speed up the dissolution, then a solution of 300 ppm ethyl acetate fraction was made. The absorbance of the fraction solution was read at a wavelength of 290 – 320 nm at 5 nm intervals, with ethyl acetate as a blank. The results of the absorbance of each fraction concentration were recorded and then the SPF value was calculated using the Mansur equation (Yulianti *et al.*, 2015). The SPF was classified and determination of the SPF number is calculated by the equation:

SPF = CF x $\sum$ (λ) x I (λ) x absorbansi (λ)

Information :

CF = Correction factor (=10)

EE (λ) = Spectrum of erythema effect

I = Intensity of the light spectrum

Abs = Absorption of sunscreen preparations

The value of EE × I is a constant value (Wungkana *et al.*, 2013; Sandi *et al*., 2021).

III. RESULT AND DISCUSSION

The sample used was 5 kg of *P. canescens (P. canescens* Jack) taken from the Madurejo Village area, Kontak makmur district, Banjar district, South Kalimantan. The liquid n-hexane fraction and the liquid ethyl acetate fraction were separated in different containers and each fraction was concentrated using a water bath to obtain a constant weight. The ethyl acetate fraction will be used as the sample in this study. The percent yield of the n-hexane fraction was 3.50% and the percent yield of the ethyl acetate fraction was 7.08%. In this study the determination of total tannin content and SPF value of *P. canescens* leaves used the ethyl acetate fraction considering that the yield obtained was higher than the n-hexane fraction. The catechin concentration series solutions were prepared at concentrations of 40 ppm, 60 ppm, 80 ppm, 100 ppm and 120 ppm from a standard stock solution of 1000 ppm and read at a maximum wavelength of 501 nm to obtain a regression equation, y = 0.002425 x + 0. 1198. The linearity value (r) obtained is 0.998.

A. Analysis of Total Tannin Ethyl Acetate Fraction

Determination of total tannin content aims to determine the tannins present in *P. canescens* leaves by UV-Vis spectrophotometry. Determination of total tannin content was measured using a standard curve of catechins with the vanillin-HCl test. Vanillin test principle-HCl in determining the content of condensed tannins is vanillin protonated in acid to form a carbocation and react with catechins. The resulting intermediate compound undergoes a dehydration reaction and produces a red colored compound . Vanillin is a quantitative test for tannins because the A ring in tannins usually has a function that can give a red chromophore with vanillin at a maximum wavelength of 500 nm. The total tannin content obtained from this study was 0.5586 ± 0.01516 b/b catechin equivalents. Percent of RSD Relative Standard Deviation) obtained is 2.7147%. The results of total tannin content can be seen in Table I.

Table I. Total Tannin Value Of *P. canescens* Concentration 300 Ppm

Abs.	Total tannin (%w/w) (QE) ±SD	Mean Total tannin (%w/w) (QE) ±SD	RSD (%)
0.762	0.589	0.558 ±0,01516	2.714
0.775	0.540		
0.821	0.578		

B. Analysis of SPF Value of the Ethyl Acetate Fraction of *P. canescens* Leaves

Determination of sunscreen activity in vitro based on the SPF value using a UV-Vis spectrophotometer at a wavelength of 290-320 nm. The existence of sunscreen activity in the ethyl acetate fraction of *P. canescens* is due to the metabolite compounds possessed by the plant, one of which is tannin whose total concentration has been measured. This compound has a chromophore group so that it can absorb light in the range of UV light wavelengths. In this study, it is included in the category of organic sunscreens that absorb UV rays such as PABA (Para-aminobenzoic acid) and oxybenzone. Fadlilaturrahmah (2021), on the ethanol extract of *P. canescens* at concentrations of 400 and 600 ppm showed an SPF value of 24 ± 0.31 and 16 ± 0.34, respectively. In this research, development was carried out in the form of the *P. canescens* fraction with a concentration of 300 ppm, and an SPF value of 5.703 ± 0.021 was obtained which indicated that sunscreen activity was categorized as moderate protection. The results obtained in the form of fractions are smaller than in the form of extracts considering that in the form of extracts, the secondary metabolites contained more compounds than in the form of fractions. Based on these results it can be seen that the concentration also affects the SPF value where the greater the sample concentration, the greater the SPF value. The results of the absorbance of the ethyl acetate fraction of *P. canescens* can be seen in Table II.

Table II. Absorbance of sample concentration 300 ppm

λ	**Abs(average)**	**A x EE x I (average)**
290	0.315	0.0047
295	0.425	0.0347
300	0.551	0.1583
305	0.555	0.1819
310	0.640	0.1192
315	0.692	0.0580
320	0.738	0.0013
Total		0.5703
SD		0.021

The SPF Value of extract *P. canescens* is higher than the fraction. Pramiastuti *et,al.* (2019), the SPF value of Etlingera Elatior ethyl acetate fraction was 2,65 ± 0,12 ppm. So, *P. canescens* has the potential to be used in the development of photo cosmetic products or the development of natural skincare.

IV. CONCLUSION

The ethyl acetate fraction of *P. canescens* leaves has a total tannin content of 0.5586 ±0.1516%w/w catechin equivalents and is an antioxidant and has

sunscreen activity based on an SPF value of 5.703 ±0.021 with moderate protection.

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CONFLICT OF INTEREST

The author declare no conflict of interest.

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