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Antioxidant Activity and Sun Protection Factor (SPF) of Various Fractions of Ethanol Extract of Kelakai Roots (*Stenochlaena palustris* Bedd)

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Abstract: Excessive sun exposure in tropical regions can cause oxidative stress that triggers premature aging and skin cancer. Kelakai root (*Stenochlaena palustris* Bedd.) is a potential natural ingredient that contains various secondary metabolites with the ability as an antioxidant and sunscreen properties. This research aims to determine the antioxidant capacity and Sun Protection Factor (SPF) value of various fractions of ethanol extract of kelakai roots. Kelakai roots are washed with clean water, then dried in a drying cabinet, and then powdered. Extraction is carried out using ethanol as a solvent using a maceration method. The extract was fractionated using n-hexane, ethyl acetate, and aqueous. Phytochemical screening was performed using specific reagents. Antioxidant capacity was determined using the DPPH method based on the Inhibitory Concentration 50 (IC50) parameter. The ability as a sunscreen is measured based on the Sun Protection Factor (SPF) parameter with a UV-Vis spectrophotometer. The results of the identification of compound groups showed that the ethyl acetate fraction contained phenolic, flavonoid, tannin, alkaloid, and saponin compounds. Antioxidant testing showed that the ethyl acetate fraction had an IC50 of 16.67 ppm (very strong), followed by the aqueous fraction IC50 of 73.92 ppm (strong) and the n-hexane fraction IC50 of 95.29 ppm (strong). At the highest concentration of 500 ppm, it is known that the n-hexane, ethyl acetate, and aqueous fractions have SPF values of 12.34, 34.62, and 15.71, respectively. The aqueous fraction is classified as medium protection, while the ethyl acetate fraction is classified as high protection, and n-hexane is classified as low protection. This study concluded that the ethyl acetate fraction of kelakai roots has strong potential as a source of antioxidants and natural sunscreen agents.
Keywords: Antioxidant; fraction; kelakai root; Sun Protection Factor; *Stenochlaena palustris.*

INTRODUCTION

Free radicals are compounds or molecules containing unpaired electrons that are reactive with other molecules. Free radicals that accumulate in large amounts in biological systems will cause oxidative stress (Chandimali et al., 2025). Oxidative stress contributes to various cancers, hypertension, diabetes mellitus, premature aging, and dementia (Papadakis et al., 2025). These various diseases remain a health problem in Indonesia that must be addressed immediately. Free radicals can originate from excessive sunlight, especially ultraviolet light. Sun exposure is particularly intense in equatorial regions with tropical climates. High-intensity sunlight over a long

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period of time can cause skin problems, including skin cancer (Tang et al., 2024). The prevalence of skin cancer worldwide in 2022 reached 146,321 cases, or 3.3%. The death rate from skin cancer reached 26,180 cases (Ferlay et al., 2021). Based on this, it is crucial to develop photoprotective agents that can prevent direct UV exposure and neutralize free radicals.

South Kalimantan has enormous potential for producing natural products. The region, largely comprised of wetlands, provides a fertile habitat for various plants (Kissinger, 2023). One plant that grows abundantly in wetlands is the kelakai (a type of plant). The kelakai plant grows wild in tidal areas and grows relatively quickly. Kelakai leaves are commonly used by the community as a vegetable or functional food to increase iron levels (Puspitasari et al., 2022). Another underutilized part is the kelakai root. Several studies have shown that the kelakai root has antioxidant properties (Adawiyah & Rizki, 2018). Kelakai root extract is known to inhibit free radicals, including its ability as a sunscreen (Paramawidhita et al., 2022).

Kelakai root contains phenolic and flavonoid compounds (Adawiyah & Rizki, 2018). Phenolic and flavonoid groups contain OH groups capable of releasing hydrogen atoms to neutralize free radicals (Bas, 2026). Phenolic and flavonoid groups also possess chromophores that absorb UV light, thereby preventing direct exposure to the skin (Zhang et al., 2023). Similar research has only examined ethanol extracts from kelakai roots. Research on fractions of ethanol extracts that demonstrate antioxidant and UV-inhibitory properties has not yet been conducted. Crude extracts comprise a complex mixture of various compound classes, rather than a single specific group. Fractions are the result of separating groups of compounds into more specific ones based on differences in polarity (Suhendy et al., 2025). Compounds are purer in fraction form, allowing the most responsible compounds to be identified and their most effective (Abubakar & Haque, 2020).

The antioxidant activity of a natural ingredient can be determined through testing using 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH testing is based on the ability of neutralizing compounds to provide electrons or hydrogen atoms to free radical compounds, especially DPPH (Gulcin & Alwasel, 2023). This method offers advantages due to its simplicity, speed, high sensitivity, and easy parameter settings. A sample's UV protection ability can be determined using the Sun Protection Factor (SPF) test. SPF testing is based on a sample's ability to absorb or scatter UV radiation before it reaches the skin (Kong, 2025). SPF testing is an in vitro model that is easily performed using a UV-Vis spectrophotometer.

The novelty of this study is that the testing was conducted on the roots of the kelakai plant, whereas most studies have examined kelakai leaves. The characteristics of secondary metabolites can differ between leaves and roots, thus providing new information. This study focused on fractions from the kelakai root extract. The resulting extract was further processed to obtain fractions using several different solvents. This process will produce fractions containing more specific compound groups. This study combines the synergistic activity of antioxidants and the interrelated SPF values. This study was conducted with the aim of determining the antioxidant capacity and SPF value of various fractions of the ethanol extract of kelakai roots.

MATERIALS AND METHODS

Equipment

The equipment used in this research includes a blender (Philips), drying cabinet (Local), rotary evaporator (IKA), water bath (Stuart), oven (Memmert), micropipette

(Dragon Lab), separating funnel (Pyrex), and UV-Vis spectrophotometer (PerkinElmer).

Materials

The materials used are kelakai root, determined by the basic laboratory of FMIPA Lambung Mangkurat University, ethanol (Technical), pro-analysis ethanol (Smartlab), sodium hydroxide (Merck), ferric chloride (Merck), Mayer's reagent (Merck), aqueous (WaterOne), gelatin (Merck), quercetin (Sigma Aldrich), n-Hexane (SmartLab), Ethyl Acetate (SmartLab), DPPH (Himedia).

Procedure

Preparation of Extract

Kelakai roots were taken from the kelakai plant growing in Sungai Pantai Village, Batola Regency, South Kalimantan. The kelakai roots were separated from the plant, washed thoroughly with running water, and rinsed with aqueous. The kelakai roots were chopped to approximately 1 cm, then drained for 60 minutes. The kelakai roots were placed in a drying cabinet at 60°C for 72 hours. The dried kelakai roots were ground into powder using a blender. The powder was extracted by maceration with ethanol for 72 hours, changing the solvent every 24 hours. The liquid extract was filtered through Whatman paper, the solvent was removed using a rotary evaporator, and the extract was oven-dried until a thick, constant-weight product was obtained (Adawiyah & Rizki, 2018).

Preparation of Fraction

Five grams of extract were dissolved in 100 mL of aqueous, then put into a separating funnel, and 200 mL of n-hexane was added, shaken for 5 minutes, and left for 15 minutes. Two layers were formed, and the n-hexane layer was taken. The water layer was then mixed with 200 mL of ethyl acetate and processed as before. The ethyl acetate layer was separated, known as the ethyl acetate fraction. The water fraction was the remaining water layer. The solvent was removed from each fraction using a rotary evaporator, and the fractions were then oven-dried until a thick fraction was obtained (Rizki et al., 2022).

Phytochemical Screening

Fifty milligrams of each fraction (n-hexane, ethyl acetate, and aqueous) was then dissolved in 20 mL of ethanol. One milliliter was then added to specific reagents for phytochemical screening. Phytochemical detection was performed for phenolics using ferric chloride solution, flavonoids using sodium hydroxide, tannins using gelatin solution, saponins using aqueous solution, alkaloids using Mayer's reagent, and steroids and terpenoids using Liebermann-Burchard reagent (Rizki et al., 2021).

Antioxidant Activity Testing

Antioxidant activity was tested on the third fraction. Four milligrams of DPPH was dissolved in ethanol to obtain a DPPH solution with a concentration of 0.4 mM. The ten milligrams of each fraction was weighed, dissolved in standard ethanol to 10 mL in a volumetric flask, and filtered through Whatman paper. These fraction solutions were then prepared at concentrations of 10, 20, 30, 40, and 50 ppm. Two mL of each fraction and concentration were taken and placed in separate test tubes. One mL of DPPH solution was added to each tube. The test tubes were shaken for 30 seconds, then allowed to stand in the dark for 36 minutes. A separate DPPH solution was prepared as a control by mixing 2 mL of standard ethanol with 1 mL of DPPH solution. The absorbance of the test and control solutions was measured using a UV-Vis spectrophotometer at 519 nm according to the results of similar research conducted by researchers (Rizki et al., 2023). The Inhibitory Concentration 50 (IC50) value was calculated (Romulo, 2020).

The IC50 value is then classified according to antioxidant strength using the categories in the Table 1.

Table 1. Antioxidant Activity Category (Rizki et al., 2022)

No	Category	Concentration (ppm)
1	Very Strong	< 50
2	Strong	50 – 100
3	Moderate	101 – 150
4	Weak	> 150

Sun Protection Factor Testing

Ten milligrams of each fraction (n-hexane, ethyl acetate, and aqueous) were dissolved separately in 100 mL of analytical ethanol. Each fraction was prepared at concentrations of 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm. The absorbance of each solution was measured at 290, 295, 300, 305, 310, 315, and 320 nm. A blank solvent of analytical ethanol was used. The absorption results are then entered into the Mansur Formula to obtain the SPF value for each concentration (Kong, 2025; Sari et al., 2022). SPF values are further categorized as shown in the Table 2. The Mansur Formula:

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

Information:

Abs = Sample Absorbance
I = Intensity Spectrum
EE = Erythemal Effect Spectrum
CF = Correction Factor + 10
Σ = The sum of the results of the multiplication of EE x I x Abs in the range of 290-320 nm with an interval of 5 nm

Table 2. Sunscreen Categories Based on SPF Value (Australia Goverment, 2023)

No	Category	SPF Value
1	Not Available	0-4
2	Low Protection	4-14
3	Medium Protection	15-29
4	High Protection	30-59
5	Very High Protection	>60

Data Analysis

In the antioxidant activity test, the absorbance was used to calculate the percentage inhibition at each concentration. Percentage inhibition is plotted against by concentration in the form of a linear regression equation to obtain the line equation y = bx + a. Substitute the value of y = 50, into the equation to obtain the x value as the IC50. In the SPF value test, the absorbance at each wavelength reading was multiplied by the EE and I values. The multiplication results were summed to obtain the total value, then multiplied by the correction factor until the SPF value was obtained. The data were averaged in Microsoft Excel, and the Standard Deviation was then calculated.

RESULTS AND DISCUSSION

Extraction and Fractionation Results

The kelakai roots used came from Sungai Pantai Village, Batola Regency, South Kalimantan. The roots were cleaned using running water to remove any

adhering soil. The presence of soil can affect the safety of kelakai roots (Hodoșan et al., 2025). The roots were dried in an oven to remove water. The presence of water is a source of microbial growth and can disrupt enzyme inactivation (Balkrishna et al., 2024). Drying of kelakai roots is done using a drying cabinet at a temperature of 60°C. This is in accordance with the Indonesian Herbal Pharmacopoeia, which states that the drying temperature of samples should not exceed 60°C (Kemenkes RI, 2022). Oven drying offers advantages such as controlled temperature, even drying, and contamination prevention. The dried roots are then ground into powder and then extracted into an extract, as seen in Figure 1.

Figure 1. Kelakai Root Powder (A) and Extract (B)

Kelakai root powder is organoleptically a dark brown powder with a distinctive rooty odor. The powdered form of the kelakai root is due to its grinding process using a blender. The purpose of making the powder is to reduce particle size and increase surface area (Shah et al., 2023). The powder readily interacts with solvents, thus facilitating the dissolution of the plant's bioactive compounds during the extraction process. The brown color is due to the drying process, which oxidizes dyes in the plant parts. The distinctive rooty odor is common in plants due to the direct exposure of these plant parts to soil (Lunz & Stappen, 2021). The kelakai root powder is then extracted using ethanol. Ethanol has the best ability to extract active compounds from the phenolic and flavonoid groups. This is due to the presence of non-polar and polar groups in the ethanol structure, so that ethanol can produce various compounds with various solubilities (Kozhantayeva et al., 2024).

The organoleptic properties of the kelakai root extract are semi-solid, brown in color, and have a distinctive root odor. The extract is semi-solid because the solvent has evaporated, leaving the bioactive material from the root. The color and odor of the root are similar to those of the powder, indicating that the extraction process did not alter the sample's organoleptic properties. The extraction results showed an extract yield of 1.25%. This result indicates the extraction of 1.25 parts of the bioactive compound from 100 parts of the sample. Other research indicates a yield of 0.04% for kelakai root extract (Handayani & Rusmita, 2017). The results of this study demonstrate a high yield. Yield is a specific parameter for plant standardization (Kemenkes RI, 2022). This parameter is specific to each plant and serves as a key indicator of the extract's identity.

Kelakai root extract was fractionated using three solvents with different polarities. Aqueous will dissolve polar compounds, ethyl acetate will dissolve semipolar compounds, and n-hexane will dissolve nonpolar compounds. The fractions of the extract will yield a more specific collection of compounds based on their polarity (Abubakar & Haque, 2020). The results of the yield obtained from the third fraction can be seen in the Table 3.

Table 3. Percentage Yield of Fractions from Kelakai Root Extract

Sample	Extract Weight	Fraction Weight	Percent Yield
n-Hexane Fraction	5 grams	1.01 g	20.2%
Ethyl Acetate Fraction	5 grams	1.52 g	30.4%
Aqueous Fraction	5 grams	1.94 g	38.8%

The fractionation results yielded the highest yield in the aqueous fraction because both organic and inorganic compounds remained in that phase. The ethyl acetate fraction had the second-highest yield because semipolar compounds tend to dissolve in ethyl acetate. Bioactive compounds from plants are generally semipolar, so they are concentrated in the ethyl acetate fraction (Indarti et al., 2019). The n-hexane fraction had the lowest yield because it binds nonpolar compounds such as steroids and terpenoids, which are not abundant in certain plants. Yield information can serve as a reference for further research, as the fraction's fractional weight is known (Coskun et al., 2021).

Phytochemical Screening Results

Phytochemical screening aims to identify the compound groups contained in the kelakai root extract fractions. Phytochemical screening is performed using specific reagents that exhibit color changes or precipitate. The results of identification of phytochemical compound groups of kelakai root extract fractions are presented in the Table 4.

Table 4. Results of Identification of Phytochemical Compound Groups in Fractions of Kelakai Root Extract

Compound Groups	Fraction		
	n-Hexane	Ethyl Acetate	Aqueous
Phenolic	+	+	+
Flavonoids	-	+	-
Tannins	-	+	+
Saponins	-	+	+
Alkaloids	-	+	-
Steroids	+	-	-
Terpenoids	+	-	-

Note: + = Positive, - = Negative

Phytochemical screening results showed differences in the composition of compound groups across fractions. The n-hexane fraction contained phenolic, steroid, and terpenoid compounds. This indicates that nonpolar compounds such as steroids and terpenoids were collected in this fraction. Phenolics have both polar and nonpolar compounds, so those found in the n-hexane fraction are nonpolar phenolics. The ethyl acetate fraction contained the most compounds, namely phenolic, flavonoid, tannin, saponin, and alkaloid compounds. The dissolved compounds were semipolar. Compounds whose structures contain polar and nonpolar groups will be more easily dissolved in ethyl acetate (Retno et al., 2025). The aqueous fraction contained phenolic, tannin, and saponin compounds. These three compounds exhibit polar-to-semipolar characteristics. Compounds of the phenolic, tannin, and saponin groups that have high polarity will be quantified in the aqueous fraction. Similar research on phytochemical screening of fractions has not been found. Information on the

identification of phytochemical compound groups of extracts indicates the presence of phenolic, flavonoid, tannin, and alkaloid compounds (Adawiyah & Rizki, 2018).

Antioxidant Activity Results

The antioxidant activity of the third fraction was measured using the DPPH method. This in vitro test modeled DPPH as a free radical. The interaction of the active compound with DPPH causes DPPH reduction, which is measured in the form of absorbance on a UV-Vis spectrophotometer (Gulcin & Alwasel, 2023). The results of the antioxidant activity test are presented in the Table 5.

Table 5. Antioxidant Activity of Fractions from Kelakai Root Extract

Sample	Replication	IC50	R^2	Average IC50 ± SD	Antioxidant Category
n-Hexane Fraction	1	96.42 ppm	0.994	95.29 ± 0.98 ppm	Strong
	2	94.61 ppm	0.994		
	3	94.84 ppm	0.992		
Ethyl Acetate Fraction	1	16.52 ppm	0.994	16.67 ± 0.45 ppm	Very Strong
	2	17.19 ppm	0.996		
	3	16.31 ppm	0.996		
Aqueous Fraction	1	72.18 ppm	0.994	73.92 ± 1.58 ppm	Strong
	2	75.27 ppm	0.994		
	3	74.33 ppm	0.992		

The three fractions showed varying antioxidant activity. The ethyl acetate fraction exhibits very strong antioxidant activity, with an IC50 value of 16.67 ppm. The R2 value ranged from 0.992 to 0.996, indicating a high correlation between concentration and percentage inhibition. There was only a 0.4–0.8% random error in the IC50 test. The presence of phenolic, tannin, and flavonoid compounds causes high antioxidant activity (Belhaoues et al., 2020). Phenolics generally contain hydroxyl groups bound to aromatic groups that can release H atoms to neutralize free radicals. The mechanism by which H atoms are released to neutralize free radicals is called hydrogen atom donation. Phenolic compounds that have lost H atoms will delocalize through resonance so that they are stable and non-reactive (Lisjak et al., 2026). Flavonoid compounds have a similar mechanism, but the presence of two OH groups in ring B, in adjacent positions, maximizes free radical neutralization (Hassanpour & Doroudi, 2023). Tannin compounds have a similar mechanism, but in biological environments, tannins can bind to prooxidant enzymes, thereby preventing oxidative stress and inactivating these enzymes (Guan et al., 2025).

The aqueous fraction has strong antioxidant activity with an IC50 value of 73.92 ppm. The IC50 value of the aqueous fraction is quite low compared to the ethyl acetate fraction. However, its ability to scavenge free radicals is still high. The presence of phenolic and tannin compounds in the aqueous fraction is the cause of this activity. The presence of tannins and phenolics is estimated to be in small amounts, so their ability is lower than that of the ethyl acetate fraction (Fitriani et al., 2025).

Furthermore, the aqueous fraction lacks flavonoids, resulting in weaker antioxidant activity. The n-hexane fraction has strong antioxidant activity with an IC50 of 95.29 ppm. The n-hexane fraction contains only phenolic, steroid, and terpenoid compounds. The detected phenolics are thought to be non-polar with weak antioxidant properties (Yahya et al., 2026). Steroids consist structurally of three cyclohexane rings and one cyclopentane ring (Dembitsky, 2024), with a few hydroxyl groups, thus exhibiting weak antioxidant properties. Terpenoids structurally have an isoprene core

skeleton in an open cyclic chain (Camara et al., 2024), so hydroxyl groups are rare, resulting in weak antioxidant activity.

SPF Measurement Results

The SPF value is one parameter used to assess a sample's ability to act as a sunscreen. The SPF value reflects the time required for protected skin to develop erythema (Tovar-Sánchez et al., 2020). The sample solution is exposed to ultraviolet light of various wavelengths using this instrument. The absorbance measurements are entered into a formula to obtain the sample's SPF value. Testing using a UV-Vis spectrophotometer has the advantages of being non-invasive, requiring no ethical protocols, being relatively fast, less expensive, and offering high precision. The results of the SPF measurements for the three fractions are presented in the Table 6.

Table 6. SPF Values of Fractions of Kelakai Root Extract

Sample	Concentration	SPF Value	Category
n-Hexane	100 ppm	3.45	Not Available
Fraction	200 ppm	6.12	Low Protection
	300 ppm	8.51	Low Protection
	400 ppm	10.63	Low Protection
	500 ppm	12.34	Low Protection
Ethyl Acetate	100 ppm	8.14	Low Protection
Fraction	200 ppm	15.72	Medium Protection
	300 ppm	21.51	Medium Protection
	400 ppm	28.68	Medium Protection
	500 ppm	34.62	High Protection
Aqueous Fraction	100 ppm	4.67	Low Protection
	200 ppm	7.81	Low Protection
	300 ppm	10.11	Low Protection
	400 ppm	13.51	Low Protection
	500 ppm	15.71	Medium Protection

According to the table, the three fractions can protect the skin from UV exposure. The ability of the three fractions differs based on the SPF value obtained. When comparing the lowest concentration in each fraction, the 100 ppm concentration in the n-hexane, ethyl acetate, and aqueous fractions has SPF values of 3.45, 8.14, and 4.67, respectively. The ethyl acetate fraction is classified as the low protection category, the n-hexane fraction as not available, and the aqueous fraction as the low protection category. At the highest concentration of 500 ppm, it is known that the n-hexane, ethyl acetate, and aqueous fractions have SPF values of 12.34, 34.62, and 15.71, respectively. The aqueous fraction is classified as medium protection, while the ethyl acetate fraction is classified as high protection, and n-hexane is classified as low protection.

The SPF values of the three fractions indicate that the ethyl acetate fraction has the highest SPF. This is due to the presence of phenolic, flavonoid, and tannin compounds. Phenolics, flavonoids, and tannins have compound structures containing chromophore groups consisting of conjugated double bonds (Stanciauskaite et al., 2022). The chromophore group has the ability to capture UV rays and then release the energy in a form that is harmless and does not damage the skin. Flavonoids have the ability to absorb UV light because they have two aromatic rings with a greater number of chromophore groups (Neugart et al., 2021). Tannins have a high structural density

due to their numerous phenolic units, which enable them to form a film layer that reflects UV light (Kriechbaum & Bergström, 2020).

Antioxidant activity and SPF values were highly correlated in this study. The antioxidant and sunscreen properties are best displayed by the ethyl acetate fraction. Phenolic, tannin, and flavonoid compounds contribute to its antioxidant properties and provide a high SPF value to the ethyl acetate fraction. The hydroxyl groups and chromophore groups present in these three groups support the antioxidant activity and sunscreen properties as photoprotectors (Jesus et al., 2023). This contrasts with the n-hexane fraction, which tends to exhibit weaker antioxidant properties and lower SPF values. This study also provides evidence that different fractions of the kelakai root ethanol extract exhibit distinct antioxidant activities and SPF values. The ethyl acetate fraction has specific properties, making it highly potential for use as an antioxidant and sunscreen (Soleimani et al., 2023). This combination of properties synergizes to maintain healthy skin and prevent skin cancer.

CONCLUSION

The ethyl acetate fraction shows very strong antioxidant activity (IC50 = 16.67 ppm), while the aqueous fraction is strong (IC50 = 73.92 ppm) and the n-hexane fraction is strong (IC50 = 95.29 ppm). At the highest concentration of 500 ppm, it is known that the n-hexane, ethyl acetate, and aqueous fractions have SPF values of 12.34, 34.62, and 15.71, respectively. The aqueous fraction is classified as medium protection, while the ethyl acetate fraction is classified as high protection, and n-hexane is classified as low protection. This study concludes that the ethyl acetate fraction of kelakai roots has the best potential as a source of antioxidants and a natural sunscreen agent.

CONFLICT OF INTEREST

The researcher has no conflict of interest in this research.

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