



**ANTIOXIDANT ACTIVITY ETHANOLIC EXTRACT OF KALAKAI LEAF
(STENOCHLAENA PALUSTRIS (BURM.F) BEDD) FROM CENTRAL
KALIMANTAN BY DPPH METHOD**

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ABSTRACT

The diseases triggered by free radicals in Indonesia are increasing. Free radicals can be neutralized by antioxidants. The main sources of antioxidants are plants or fruits. Kalakai (*Stenochlaena palustris* (Burm.f.) Bedd.) is one of the typical plants of Kalimantan known for its medicinal properties. The leaves of Kalakai contain compounds such as flavonoids, phenols, tannins, alkaloids, and saponins, which have antioxidant abilities. This research aims to determine the antioxidant activity of Kalakai leaf extract from Central Kalimantan against DPPH free radicals based on the IC₅₀ value. Antioxidant activity was measured using the DPPH method and analyzed using UV-Vis spectrophotometry at a wavelength of 516.6 nm. The results showed that Kalakai leaf extract had moderate antioxidant activity with an IC₅₀ value of 241.547 ppm, while vitamin C as a positive control had strong activity with an IC₅₀ of 10.031 ppm. These findings indicate that Kalakai leaves, although less potent than vitamin C, may serve as a promising natural antioxidant source. The conclusions is kalakai leaf extract exhibits moderate antioxidant activity and holds potential for further development in natural antioxidant applications.

Keywords: antioxidant; DPPH; IC₅₀; kalakai leaf; *stenochlaena palustris*

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INTRODUCTION

In the modern era, with the advancement of technology and science, there has been a change in the lifestyle of society which has adverse effects on health, such as consumption of unbalanced nutrition, lack of exercise and rest, smoking habits, and alcohol consumption. Furthermore, deteriorating environmental conditions, such as pollution, also contribute to a decline in the quality of life due to the reduction in the production of compounds that maintain body condition, namely natural antioxidants used to neutralize free radicals formed due to air pollution, radiation sources, harmful chemicals, and the formation of other free radicals (Arnanda & Nuwarda, 2019). The damages caused by free radicals can be inhibited by antioxidant compounds. Antioxidant compounds neutralize, reduce, and inhibit the formation of new free radicals in the body by donating electrons to free radicals, thus turning them into paired free electrons and stopping damage in the body. Antioxidants can be produced endogenously or obtained exogenously to help neutralize free radicals present in the body (Adawiyah et al., 2023).

Kalimantan is an island in Indonesia known for its rich biodiversity. Not only that, but the wealth of traditional medicinal knowledge using plants passed down orally from generation to generation among the native ethnic groups of Kalimantan is also abundant (Fahruni, 2018). One of the typical plants of Kalimantan known for its medicinal properties is Kalakai (*Stenochlaena palustris* (Burm.f.) Bedd.). Kalakai is a plant commonly consumed by people in their daily lives. It is easily found in the Kalimantan region, especially in areas inhabited by Dayak communities. Dayak people believe that consuming Kalakai leaves can treat diseases such as anemia, and for breastfeeding mothers, Kalakai leaves are believed to increase and facilitate breast milk production. Based on the

research by Okfrianti (2022) phytochemical screening results on ethanol extracts of Kalakai leaves contain flavonoids, phenols, tannins, alkaloids, and saponins. In Al-snafi (2021) the antioxidant activity test on Kalakai leaf extracts from East Kalimantan using the DPPH method showed that the ethanol extract of Kalakai leaves had an IC₅₀ value of 143.1431 ppm, categorized as moderate antioxidants, with ethanol 70% used as the testing solvent. Meanwhile, in Rifqi (2022), the antioxidant activity test of ethanol extracts of Kalakai leaves from East Kalimantan using the DPPH method resulted in an antioxidant activity value of 51.86 µ/ml, classified as strong antioxidants, with ethanol p.a 96% used as the testing solvent. Furthermore, there was a previous study by Adawiyah & Rizki (2018) on the antioxidant activity test of ethanol extracts of Kalakai roots (*Stenochlaena palustris* Bedd) from Central Kalimantan, which compared the antioxidant levels of Kalakai roots grown in peat and sandy soils. The results showed that the antioxidant activity of Kalakai roots in peat soil was stronger, with an IC₅₀ value of 19.06 ppm, compared to Kalakai root extracts in sandy soil with an IC₅₀ value of 24.40 ppm. Based on the above description, there has been no research on antioxidant activity tests for Kalakai leaves originating from Central Kalimantan using the DPPH method. Therefore, we interested in conducting research on the antioxidant activity of Kalakai leaves originating from Central Kalimantan.

METHOD

This research was conducted using quantitative methods and laboratory experimental research in vitro, which is analytical in nature, to determine the antioxidant activity of ethanol extract from Kalakai leaves. The equipment used in this research includes an analytical balance (Pioneer PA214C, Ohaus, US), chemical glassware (Pyrex) 50 mL, 500 mL, and 1000 mL, Erlenmeyer flasks (Pyrex), containers, glass funnels, blender (Philips), test tube rack, test tubes, stirring rods, spatula, watch glass, UV-Vis spectrophotometer (Shimadzu type UV-1700), cuvettes, measuring flasks 25 mL & 50 mL, measuring pipettes, ball pipettes, micropipettes (Socorex), porcelain dishes, water bath (Mettler, WNB 14 Ring, Germany), and rotary vacuum evaporator (IKA RV10, Germany). The materials used in this research are Kalakai leaf *simplicia*, 70% ethanol, p.a. ethanol, DPPH (2,2-diphenyl-1-picrylhydrazyl), vitamin C, aluminum foil, cotton, filter cloth, and filter paper. The materials used in this research are fresh young Kalakai leaves, green and reddish in color, taken from Jalan Mahir Mahar, Kelurahan Kalampangan, Palangka Raya City. Then, sorting was done to obtain good Kalakai leaves, followed by wet sorting, drying at room temperature away from direct sunlight, and then dry sorting before blending until smooth. The extraction process using the maceration method involved weighing 877 grams of powder, followed by extraction using 70% ethanol solvent with a ratio of 1:5 for 3x24 hours with solvent replacement every 24 hours. The liquid extract was then separated from the residue using filter paper. Re-maceration was performed twice. The liquid extract obtained was concentrated using a rotary vacuum evaporator at low pressure at a temperature not exceeding 70°C, then evaporated on a water bath at 60°C until a thick extract was formed (Faizal et al., 2020)

Antioxidant Activity Test

1. Preparation of 0.4 mM DPPH Solution
Weigh 7.9 mg of DPPH powder using an analytical balance and transfer it into a 50 mL measuring flask. Then, add p.a. ethanol until the mark.
2. Determination of DPPH Maximum Wavelength
Take 0.5 mL of 0.4 mM DPPH and transfer it into a reaction tube. Add 2 mL of p.a. ethanol, mix well, and let it stand for 30 minutes in the dark. Measure the absorbance of the solution using a UV-vis spectrophotometer at wavelengths ranging from 450 nm to 600 nm. Repeat this process three times.
3. Determination of DPPH Operating Time
Take 0.5 mL of 0.4 mM DPPH and transfer it into a reaction tube. Add 2 mL of 2 ppm vitamin C. Read the absorbance at the maximum wavelength at 2-minute intervals for 60 minutes (Patria & Soegihardjo, 2013).
4. Preparation of Sample Stock Solution (1000 ppm):

Weigh 50 mg of Kalakai leaf extract and transfer it into a 50 mL measuring flask. Then, add p.a. ethanol until the mark to obtain a concentration of 1 mg/mL. Cover the flask with aluminum foil (Susanti et al., 2021).

5. Determination of IC₅₀ Value of Kalakai Leaf Extract Sample Solution:

Prepare sample stock solution concentrations of 20, 40, 60, 80, and 100 ppm. Take 0.5 mL of 0.4 mM DPPH and transfer it into a reaction tube. Then, add various concentrations of sample solution serially into each reaction tube. Let the solution stand for 30 minutes and measure its absorbance at the maximum wavelength of 516.6 nm. Repeat this process three times for each concentration (Damanis et al., 2020).

6. Preparation of Positive Control Stock Solution (Vitamin C) as a Comparator (100 ppm):

Weigh 5 mg of vitamin C and transfer it into a 50 mL measuring flask. Then, add p.a. ethanol until the mark (Okfrianti et al., 2022).

7. Determination of IC₅₀ Value of Positive Control Solution (Vitamin C) as a Comparator:

Prepare positive control solutions with serial concentrations of 2, 4, 6, 8, and 10 ppm. Take 0.5 mL of 0.4 mM DPPH and transfer it into each reaction tube. Then, add 2 mL of various concentrations of the standard positive control solution into each reaction tube. Let the solution stand for 30 minutes in the dark and measure its absorbance at the maximum wavelength of 516.6 nm. Repeat this process three times for each concentration (Patria & Soegihardjo, 2013). Manufacture of a positive control parent solution (Vitamin C) as a comparator (100 ppm)

Data Analysis

Data processing was conducted using Microsoft Excel to obtain a linear regression equation, which was then utilized for data analysis. The smaller the IC₅₀ value, the stronger the antioxidant activity. Subsequently, the IC₅₀ results were matched with the parameters of free radical scavenging activity. These parameters are presented in Table 1.

Table 1.
Antioxidant Activity Based on IC₅₀ Values (Adawiyah & Rizki, 2018)

Activity	IC ₅₀ Value
Very Active	<50 ppm
Strong	50-100 ppm
Moderate	101-250 ppm
Weak	250-500 ppm
Not Strong	>500 ppm

The absorbance (abs) results were analyzed using the equation (Rizki et al., 2021):

$$\% \text{Inhibition} = \frac{[\text{abs DPPH} - \text{abs sample}]}{(\text{abs DPPH})} \times 100 \%$$

IC₅₀ values were categorized based on the antioxidant activity categories generated from the samples. The calculation of these values was based on the linear regression equation: $y = bx + a$.

RESULT

Extraction of Ethanol Extract from Kalakai Leaves

The powdered plant material was subjected to extraction using the maceration method. The maceration process was carried out using 70% ethanol solvent for 3x24 hours, with solvent replacement every 24 hours. The resulting liquid extract was then evaporated using a rotary vacuum evaporator at low pressure at a temperature not exceeding 70°C. The treatment with the evaporator aimed to separate the extract from its solvent to obtain a concentrated extract. This concentrated extract was subsequently evaporated on a water bath at 60°C until a thick extract was formed, with a yield of $29.7008 \pm 3.4\%$.

Antioxidant Activity Test

The antioxidant activity was assessed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method. This method was chosen due to its simplicity, ease of use, rapidity, and

requirement for minimal sample (Widyasanti et al., 2016). Ethanol p.a. was used as the solvent in this study as DPPH can dissolve in organic solvents with polar properties. The antioxidant activity test was conducted using a UV-Vis spectrophotometer. The maximum wavelength obtained in this study was 516.6 nm, which theoretically matches the wavelength range of 515 nm to 520 nm for measuring DPPH, as reported by (Faizal et al., 2020). Subsequently, the operating time was determined. The operating time was optimized within the range of 0-60 minutes. In this study, the absorbance stabilized between minutes 26 and 32, and instability occurred after minute 32, indicating compound degradation. According to references, the operating time for DPPH is at minute 30, which aligns with the operating time found in this study (Patria & Soegihardjo, 2013).

The IC₅₀ value was defined as the concentration of the test compound required to scavenge 50% of free radicals. The lower the EC₅₀ or IC₅₀ value of a test compound, the more effective it is as a free radical scavenger (Faizal et al., 2020). The antioxidant activity test of the test compounds was replicated three times. According to Huselan (2015), a compound is considered to be highly active as an antioxidant if the IC₅₀ value is less than 50 ppm, strong if the IC₅₀ value is 50-100 ppm, moderate if the IC₅₀ value ranges from 101-250 ppm, weak if the IC₅₀ value ranges from 250-500 ppm, and not strong if the IC₅₀ value exceeds 500 ppm. Before conducting the antioxidant activity test on the samples, the absorbance of the blank was measured. The blank absorbance obtained in this study was 0.815 ppm.

Antioxidant Activity Test of Vitamin C

Vitamin C was used as the positive control in this test. The use of a positive control in the antioxidant activity test was to determine the antioxidant potential of the ethanol extract from Kalakai leaves compared to vitamin C. Vitamin C was chosen as the reference because it is a natural antioxidant commonly used as a reference compound in antioxidant activity tests due to its safety and lack of toxicity. The absorbance of vitamin C was measured in a series of concentrations: 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. The purpose of creating this concentration series was to determine the IC₅₀ value using a linear regression mathematical equation. Linear absorption can be provided if at least five increasing concentration standard ranges are created (Rizki et al., 2022). The results of the antioxidant activity test of vitamin C as a positive control using the DPPH method with a UV-Vis spectrophotometer can be observed in the table below.

Table 2.

Vitamin C Absorbance Results

Compound	Concentration (ppm)	Absorbance (replication)			Average ± SD
		1	2	3	
Vitamin C	2	0,760	0,761	0,761	0,760± 0,001
	4	0,672	0,671	0,671	0,671± 0,001
	6	0,593	0,593	0,593	0,593± 0,000
	8	0,492	0,492	0,492	0,492± 0,000
	10	0,410	0,409	0,409	0,409± 0,001

Table 3.

Percent Results of Vitamin C Inhibition

Compound	Concentration (ppm)	Percent Inhibition (%)			Average ± SD
		1	2	3	
Vitamin C	2	6,748	6,626	6,626	6,667 ± 0,070
	4	17,546	17,669	17,669	17,628 ± 0,071
	6	27,239	27,239	27,239	27,239 ± 0,000
	8	39,632	39,632	39,632	39,632 ± 0,000
	10	49,693	49,816	49,816	49,775 ± 0,071

Based on the results obtained, it can be observed that the higher the concentration (ppm) of vitamin C, the higher its scavenging activity against free radicals. According to Patria & Soegihardjo (2013), as the concentration increases, the percentage of free radical inhibition (DPPH) also increases. This phenomenon occurs because more hydrogen atoms from the hydroxyl groups are provided to the DPPH radical, resulting in the reduction of DPPH to DPPH-H, indicated by a color

change from purple to yellow. Additionally, vitamin C has two hydroxyl groups, making it easier to donate its hydrogen atoms. The relationship between concentration and percentage inhibition of vitamin C is depicted in Figure 1, Figure 2, and Figure 3.

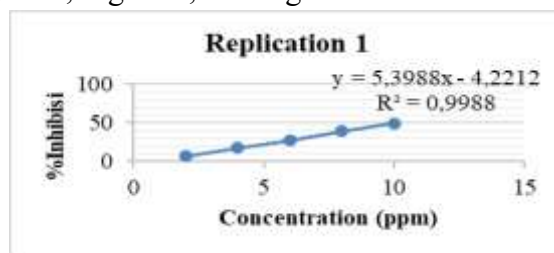


Figure 1. First Replication Between Concentration and Percent Inhibition of Vitamin C

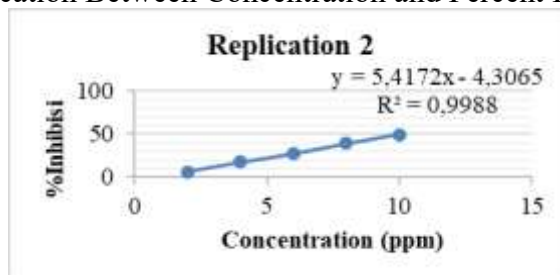


Figure 2. Second Replication Between Concentration and Percent Inhibition of Vitamin C

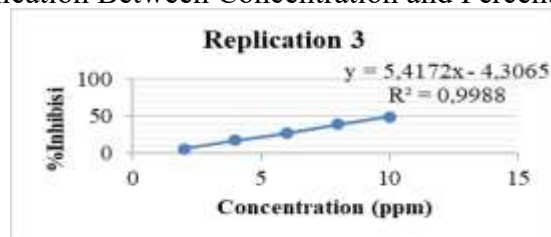


Figure 3. Third Replication Between Concentration and Percent Inhibition of Vitamin C

The equation was derived based on the graph between the independent variable, concentration of the solution (x), and the dependent variable, percentage inhibition (y). The equation yielded a correlation coefficient from 3 replications, which is $(r) = 0.998$. A correlation value (r) of 0.998 for vitamin C indicates a perfect correlation. The value of $(r) = 0.998$, which is close to +1 (positive value), signifies that as the concentration of vitamin C increases, its antioxidant activity also increases. From the linear equation obtained, the IC₅₀ value of vitamin C can be calculated. The IC₅₀ value of vitamin C can be seen in Table IV.

Table 4.
IC₅₀ Vitamin C results

Compound	IC ₅₀ (ppm)			Average ± SD
	1	2	3	
Vitamin C	10,043	10,025	10,025	10,031 ± 0,011

The average IC₅₀ value of vitamin C is 10.031 ppm, classifying it as a very strong antioxidant because it has an IC₅₀ value of less than 50 ppm. The lower the IC₅₀ value, the stronger the antioxidant activity. This result also validates the method used in antioxidant testing because theoretically, vitamin C has strong activity against free radicals.

Antioxidant Activity Test of Ethanol Extract from Kalakai Leaves

The concentration series of ethanol extract from Kalakai leaves in this study were 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. The results of the antioxidant activity test of the Kalakai leaf extract can be observed in the table 5.

Table 5.
Absorbance Results of Kalakai Leaf Ethanol Extract

Compound	Concentration (ppm)	Absorbance (replication)			Average \pm SD
		1	2	3	
Kalakai Leaf Ethanol Extract	20	0,808	0,808	0,808	$0,808 \pm 0,000$
	40	0,795	0,795	0,795	$0,795 \pm 0,000$
	60	0,760	0,761	0,760	$0,760 \pm 0,001$
	80	0,704	0,703	0,703	$0,703 \pm 0,001$
	100	0,667	0,667	0,667	$0,667 \pm 0,000$

Table 6.
Results of Percent Inhibition of Kalakai Leaf Ethanol Extract

Compound	Concentration (ppm)	Absorbance (replication)			Average \pm SD
		1	2	3	
Kalakai Leaf Ethanol Extract	20	0,859	0,859	0,859	$0,859 \pm 0,000$
	40	2,454	2,454	2,454	$2,454 \pm 0,000$
	60	6,748	6,626	6,748	$6,707 \pm 0,070$
	80	13,620	13,742	13,742	$13,701 \pm 0,070$
	100	18,160	18,160	18,160	$18,160 \pm 0,000$

Based on the results obtained, it can be observed that the higher the concentration (ppm) of ethanol extract from Kalakai leaves, the higher its scavenging activity against free radicals. The relationship between concentration and percentage inhibition of the ethanol extract from Kalakai leaves is depicted in Figure 4, Figure 5, and Figure 6.

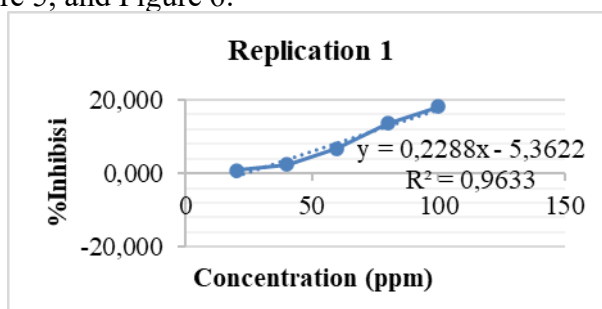


Figure 4. Replication 1 of the Relationship Between Concentration and Percent Extract Inhibition Kalakai Leaf Ethanol

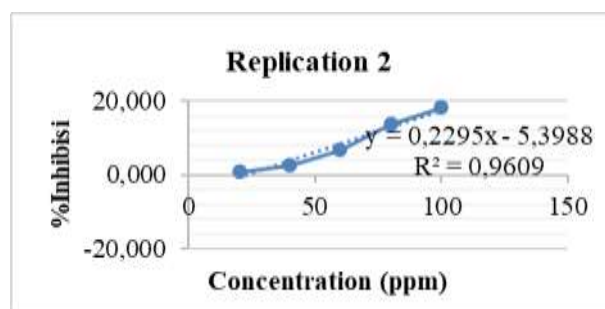


Figure 5. Replication of 2 Relationships Between Concentration and Percent Inhibition of Kalakai Leaf Ethanol Extract

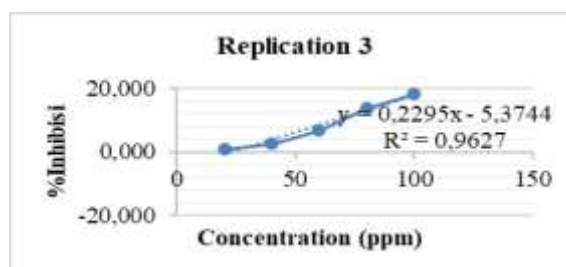


Figure 6. Replication of 3 Relationships Between Concentration and Percent Inhibition of Kalakai Leaf Ethanol Extract

The equation yielded correlation coefficients from 3 replications as follows: replication 1 (r) = 0.9633, replication 2 (r) = 0.9609, and replication 3 (r) = 0.9627. The correlation coefficient values (r) for the ethanol extract from Kalakai leaves indicate a perfect correlation. The correlation coefficient values obtained, approaching +1 (positive value), indicate that as the concentration of the ethanol extract from Kalakai leaves increases, its antioxidant activity also increases. From the linear equation obtained, the IC₅₀ value of the ethanol extract from Kalakai leaves can be calculated. The IC₅₀ value can be observed in Table 7.

Tabel 7.
IC₅₀ Results of Ethanol Extract of Kalakai Leaves

Compound	IC ₅₀ (ppm)			Average ± SD
	1	2	3	
Ekstrak Etanol Daun Kalakai	241,968	241,389	241,283	241, 547 ± 0,369

DISCUSSION

From the obtained linear equation, the average IC₅₀ value of the ethanol extract from Kalakai leaves is 241.547 ppm, categorizing it as a moderate antioxidant because it falls within the range of 101-250 ppm. This finding is consistent with the study conducted by Okfianti (2022), which reported an antioxidant activity with an IC₅₀ value of 143.1431 ppm, also classified as a moderate antioxidant. However, it differs from the study by Adawiyah (2023), which reported an IC₅₀ value of 51.86 µ/ml for the Kalakai leaf extract, categorizing it as a strong antioxidant. This study proves that the ethanol extract from Kalakai leaves possesses antioxidant activity, which is attributed to its chemical compounds such as flavonoids, phenolic compounds, tannins, alkaloids, and saponins. The difference in IC₅₀ values obtained in this study compared to others is due to the extract's ability to donate electrons to the DPPH radical, and phenolic compounds play a significant role in the antioxidant activity of the sample. The higher the phenolic content in the extract, the greater the potential of the sample as a natural antioxidant. Additionally, other factors affecting the IC₅₀ value of the Kalakai leaf extract include the antioxidant activity testing technique, solvent, concentration used, as well as the location where the plant was harvested, leading to variations in the chemical composition of the Kalakai plant.

CONCLUSION

Based on the research findings, it can be concluded that the ethanol extract from Kalakai leaves originating from Central Kalimantan possesses antioxidant activity. The obtained IC₅₀ value for the ethanol extract from Kalakai leaves is 241.547 ppm, which falls into the moderate category with IC₅₀ values ranging from 101 to 250 ppm.

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