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Determination of Total Flavonoid Content and Antioxidant Activity Test of Kelakai Stem and Leaf Extract (*Stenochlaena palustris* (Burm. F) Bedd)

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ABSTRACT

Kelakai plants belong to the Polypodiaceae family and originate from Central Kalimantan. The increasing prevalence of diseases related to oxidative stress has led people to consume natural antioxidants derived from plants. Kelakai plants exhibit antioxidants, anti-inflammatory, and anti-hyperlipidemia activities. Based on these factors, researchers aim to examine the total flavonoid content and determine the antioxidant activity of ethanol extract from the stems and leaves of the kelakai plant (*Stenochlaena palustris* (Burm. F) Bedd). It is hoped that this study can be an important part of exploring antioxidant compounds obtained from natural materials around us. This study aims to determine the total flavonoid content and understand the antioxidant activity of ethanol extract of Kelakai stems and leaves (*Stenochlaena palustris* (Burm. F) Bedd). The total flavonoid content was tested using the UV-Vis spectrophotometer method with quercetin standard and expressed in ppm. Meanwhile, the antioxidant activity was determined using the DPPH method, with results expressed in Inhibition Concentration 50% (IC50). The results of the total flavonoid content of ethanol extract were 76.727±0.497 ppm, and the antioxidant activity of ethanol extract of kelakai stems and leaves (*Stenochlaena palustris* (Burm. F) Bedd) had an IC50 value of 78.787±0.103 ppm, indicating strong intensity and potential as a source of natural antioxidants containing flavonoid compounds.

Keywords: Stenochlaena palustris (Burm.) Bedd, total flavonoid, antioxidant

Introduction

Indonesia is rich in natural resources with various plant species spread across the country. In Manado, Kalimantan, Java, and other regions, plants are still used as traditional medicine, passed down from generation to generation through simple processing methods (Adiyasa & Meiyanti, 2021). The benefits of plants include disease prevention and treatment, as well as immune system enhancement (Susi & Nurbaeti, 2015).

Flavonoids are compounds most commonly found in plants that are beneficial to health, exhibiting activities as anticancer, anti-inflammatory, antioxidant, and anti-allergic agents, and can prevent atherosclerosis (Nugroho, 2017). Flavonoid compounds have the potential as antioxidants. Flavonoids can be found in plants that are beneficial to the human body, such as the Kelakai plant (*Stenochlaena palustris* (Burm.F) Bedd). This plant originates from Central Kalimantan and is commonly consumed by people, either sautéed or boiled. The antioxidant content in the plant acts as a radical scavenger and helps convert free radicals into less reactive forms (Savitri et al., 2021).

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The increasing prevalence of diseases related to oxidative stress has led people to consume natural antioxidants derived from plants. Natural antioxidants in food can neutralize or prevent excessive free radicals in our bodies (Pratiwi et al., 2022) and can also improve the quality of people's health at affordable costs.

Based on these factors, researchers aim to examine the total flavonoid content and determine the antioxidant activity of ethanol extract from the stems and leaves of the Kelakai plant (*Stenochlaena palustris* (Burm. F) Bedd). It is hoped that this study can be an important part of exploring antioxidant compounds obtained from natural materials around us.

Material and Methods

Equipment

The equipment used in this study includes a UV-visible spectrophotometer, rotary evaporator, oven, macerator, water bath, electric stirrer, blender, micropipette, analytical balance, mesh sieve (size 60), a set of glassware (Pyrex®), Büchner funnel, test tube rack, porcelain dish, test tube clamp, metal spatula, and spray bottle.

Materials

The materials used in this study are powdered Kelakai leaf simplicia, 70% ethanol, 1 M potassium acetate, 10% aluminum chloride, quercetin, Meyer's reagent, Bouchardat's reagent, Dragendorff's reagent, 1% FeCl3, 2 N HCl, concentrated HCl, concentrated H2SO4, chloroform, acetic acid anhydride, Mg powder, aluminum foil, and filter paper.

Subsection 1

The method is sample collection: kelakai

The study was conducted at the Biology Laboratory of Ahmad Dahlan University, Yogyakarta.

a. Sample Collection: Kelakai stems and leaves were obtained from Palangka Raya, Central Kalimantan, with characteristics of young stems and leaves. Determination was performed to ensure that the plant used was Kelakai. Then the Kelakai stems and leaves were sorted and dried in the sun covered with black cloth until dry and easily broken, then blended and sieved using a size 60 mesh.
b. Preparation of Sample Extract Using Maceration Method with 70% Ethanol. This was done once for 24 hours with two repetitions and protected from sunlight. The ethanol extract obtained was then concentrated using a rotary evaporator to produce a thick brown extract.

Subsection 2

The method is described: determination of total flavonoid content using UV-Vis spectrophotometer method:

a. Standard Quercetin Stock Solution (400 ppm): Accurately weigh 10 mg and place it in a 25 mL volumetric flask, dissolve it in ethanol, sonicate, and then add ethanol until it reaches the mark.
b. Determination of Operating Time: Take 0.5 mL of 50 ppm quercetin solution, place it in a 5 mL volumetric flask, add 1.5 mL of ethanol, 0.1 mL of 10% aluminum chloride, 1 mL of 1 M sodium acetate, and add purified water (aquabidest) to the mark. Measure at a wavelength of 438 nm for 3600 seconds.
c. Determination of Sample Content: Weigh 250 mg of the sample, place it in an Erlenmeyer flask, add 2 mL of ethanol, vortex, and sonicate for 1 hour. Centrifuge, take the filtrate, and place it in a 10 mL volumetric flask. Repeat extraction 3 times. Add ethanol to the mark. Sample dilution (FP) 2X.
d. The flavonoid content obtained is then entered into the absorbance data using the quercetin curve as the y value and the quercetin concentration as the x value.

Subsection 3

The method is described: determination of the antioxidant activity of ethanol extract of kelakai stems and leaves:

a. Preparation of DPPH Reagent (0.15 mM): 20 mg DPPH was dissolved in 10 mL of ethanol p.a., placed in a 50 mL volumetric flask, ethanol p.a. was added to the mark, and homogenized.
b. Preparation of Sample Solution: 250 mg, sonicated for 15 minutes then filtered.
c. Measure the wavelength from 450-600 nm using a UV-Vis spectrophotometer.
d. Determination of Antioxidant Activity: 1 mL of each concentration of test solution was placed in a test tube, 1 mL of DPPH was added and homogenized using a vortex, then incubated at the operating time range at room temperature and the absorbance was measured at the maximum wavelength obtained.
e. The percentage of inhibition was calculated as the percentage of reduction in DPPH color using the formula:
   % inhibition = $\frac{A1-A2}{A1}$x 100 %
f. The IC_{50} value is between the concentration of the test solution (x-axis) and the % inhibition (y-axis) from the equation y = a + bx, and the IC_{50} value can be calculated using the formula:
   IC_{50} =$\frac{50-a}{b}$

Data analysis

Antioxidant activity is obtained from the calculation of X in the linear regression equation obtained from the percentage of inhibition (Hikmah & Anggarani, 2021):

$$\text{DPPH Inhibition} = \frac{Abs\ blanko - abs\ sample}{Abs\ blanko} \text{x } 100\ \%$$

The percentage of inhibition of antioxidant activity from various extract concentrations and quercetin is made into a linear regression equation. Where the x-axis is the sample concentration and the y-axis is the percentage of inhibition of antioxidant activity, so y=ax+b. The IC_{50} value is calculated when the percentage of antioxidant activity is 50%, which is the concentration of the solution capable of providing 50% DPPH scavenging (Molyneux, 2003).

Results and Discussion

Kelakai determination results: kingdom (*plantae*), division (*pteridophyte*), class (*pteridopsida*), order (*polypodiales*), family (*polypodiaccae*), genus (*stenochlaena*), species (*Stenochlaena palustris* (Burm,F) Bedd)

Kelakai stems and leaves simplisia (*Stenochlaena palustris* (Burm. F) Bedd)) that has been macerated produced a thick extract of 96.840 grams with a yield of 9.46%. Maceration is the simplest extraction method, and has the advantage of low cost, simple equipment, and without heat treatment, making it the right choice for the extraction of heat-sensitive (thermolabile) compounds (Nugroho, 2017).

Table 1. Results of ethanol extract of kelakai stems and leaves

Sample	Simplicia	Thick Extract	Yield (%)
Kelakai stems and leaves	1023 g	96.840 g	9.46 %

The use of quercetin as a standard reference is because quercetin is one of the flavonoid glycoside groups commonly found in plant species (Wahid et al., 2022). Flavonoids are compounds that are yellow (Nangoy et al., 2019).

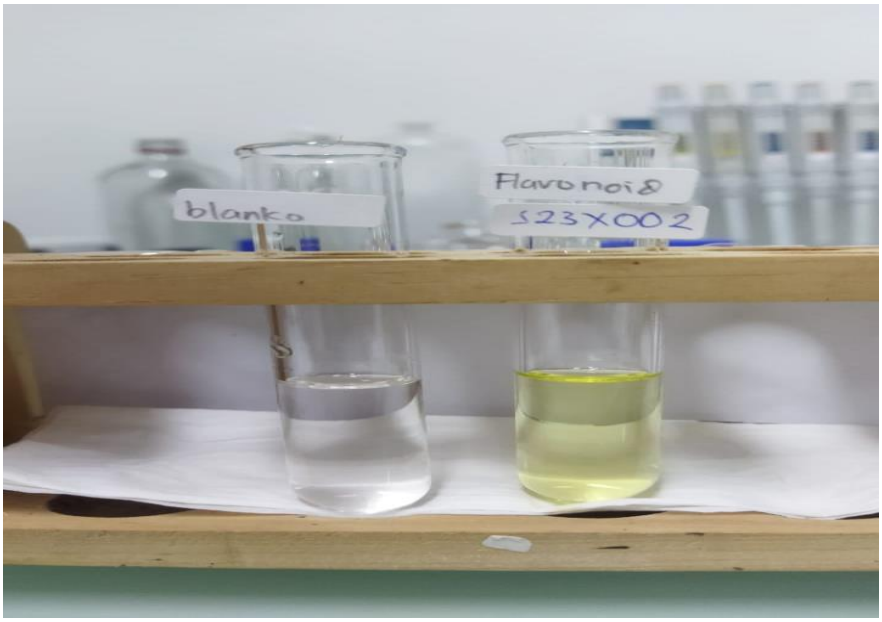

Figure 1. Flavonoid test

Based on the standard curve, obtained y = 0.00804x + 0.00444 with a correlation value (r) of 0.99984. The maximum wavelength of quercetin obtained is 438 nm for 3600 seconds. From the standard quercetin measurement data and absorbance data, it is shown that the higher the concentration, the higher the absorbance obtained. The absorbance value depends on the concentration of the substance contained in it, therefore the higher the concentration of the substance contained in a sample, the more molecules will absorb light at a certain wavelength.

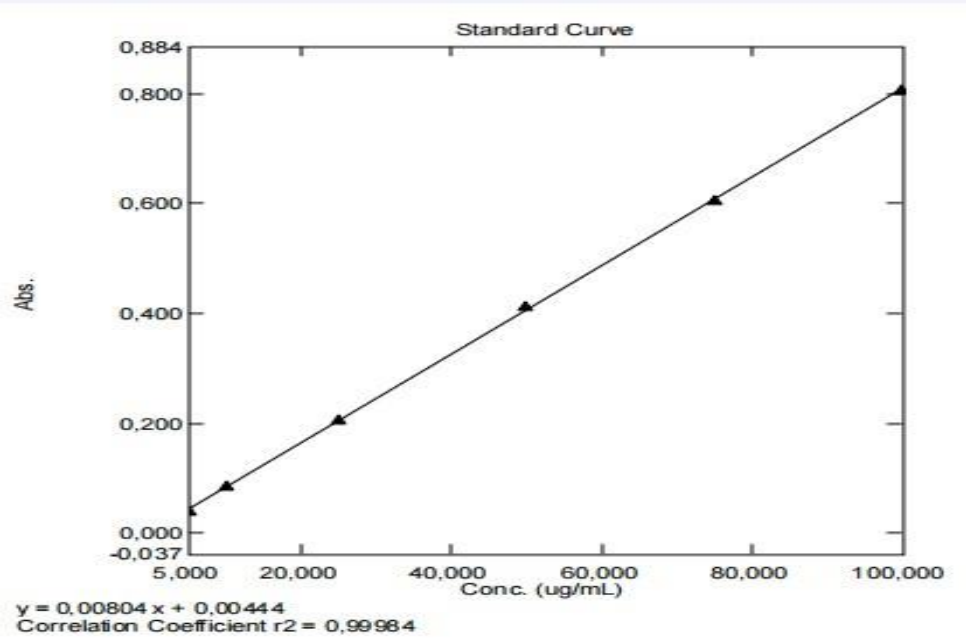

Figure 2. Linear regression graph of standard quercetin

The total flavonoid content of the ethanol extract of Kelakai stems and leaves are 76.727±0.497 ppm. This is by the study by Syamsul et al. (2019) that *Stenochlaena palustris* (Burm.F) Bedd) contains flavonoids.

Table 2. Results of total flavonoid content determination

Replication	Absorbance (y)	Concentration	Mean flavonoid content (ppm)
I	0.311	38.152	
II	0.313	38.363	76.727±0.497
III	0.315	38.625	

The test solution of the stem and leaf extracts of Kelakai, which has been added to the DPPH solution, was placed into a test tube and its absorbance was measured at a maximum wavelength of 517 nm.

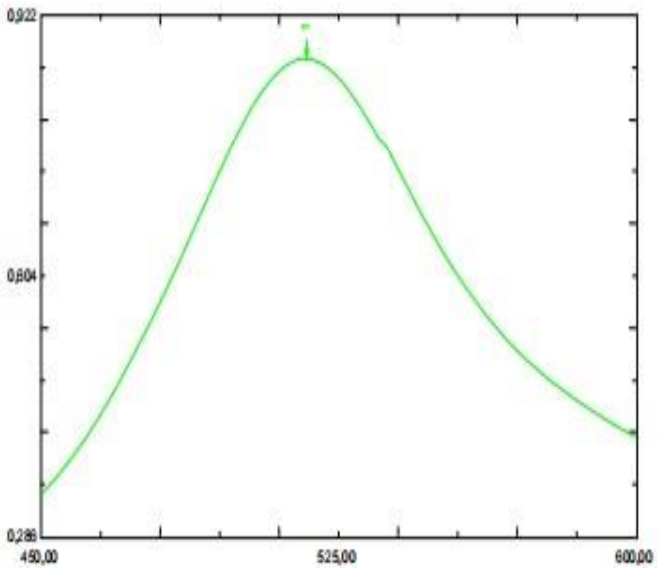

Figure 3. Negative control

In this study, a color change from dark purple to pale yellow. According to Jami'ah et al. (2018), if there is a color change from dark purple to yellow at each tested concentration, it indicates antioxidant activity.

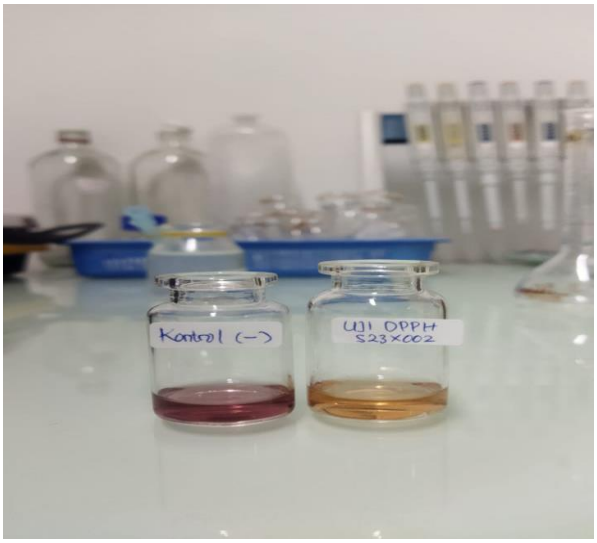

Figure 4. The color of ethanol extract of Kelakai stem and leaf after DPPH inhibition

From the data y = -0.00654x + 0.94963 = 0.99660, it was found that the linear regression results showed a good correlation. Thus, with the increase in concentration of the ethanol extract of Kelakai stems and leaves, the antioxidant capacity also increases.

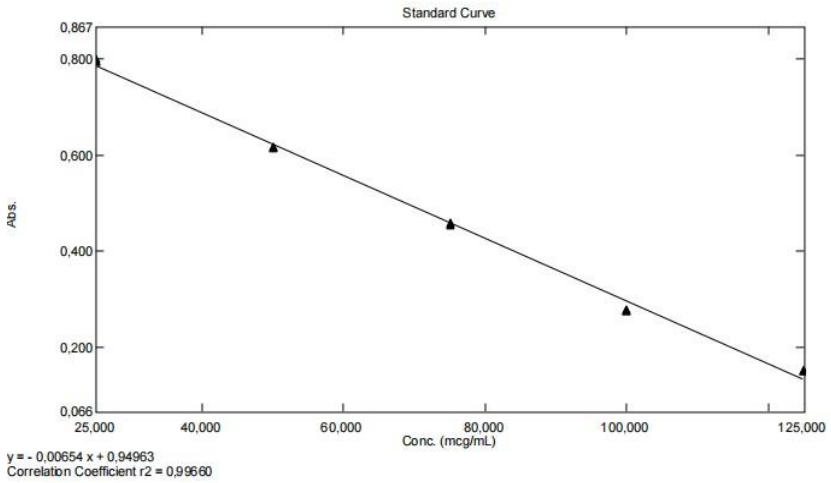

Figure 5. DPPH Regression

This indicates that the ethanol extract of Kelakai stems and leaves possesses antioxidant activity. Subsequently, the absorbance of the ethanol extract of Kelakai stems and leaves at each concentration is measured to determine the percentage of inhibition.

Table 3. Data of concentration and absorbance of ethanol extract of kelakai stem and leaves

con	abs	control	% inhibition	Average
125	0.152	0.869	82.509	82.700
125	0.15	0.869	82.739	
125	0.149	0.869	82.854	
100	0.274	0.869	68.470	68.278
100	0.277	0.869	68.124	
100	0.276	0.869	68.239	
75	0.458	0.869	47.296	47.488
75	0.454	0.869	47.756	
75	0.457	0.869	47.411	
50	0.617	0.869	28.999	29.037
50	0.616	0.869	29.114	
50	0.617	0.869	28.999	
25	0.8	0.869	7.940	8.247
25	0.795	0.869	8.516	
25	0.797	0.869	8.285	

The IC_{50} value obtained from the ethanol extract of Kelakai stems and leaves is 78.787±0.103 ppm. The categories for the strength of antioxidant compounds are as follows: <50 very strong, 50 - 100 strong, 100 - 250 moderate, 250 - 500 weak, and >500 inactive (Molyneux, 2004, as cited in Irianti et al. (2017).

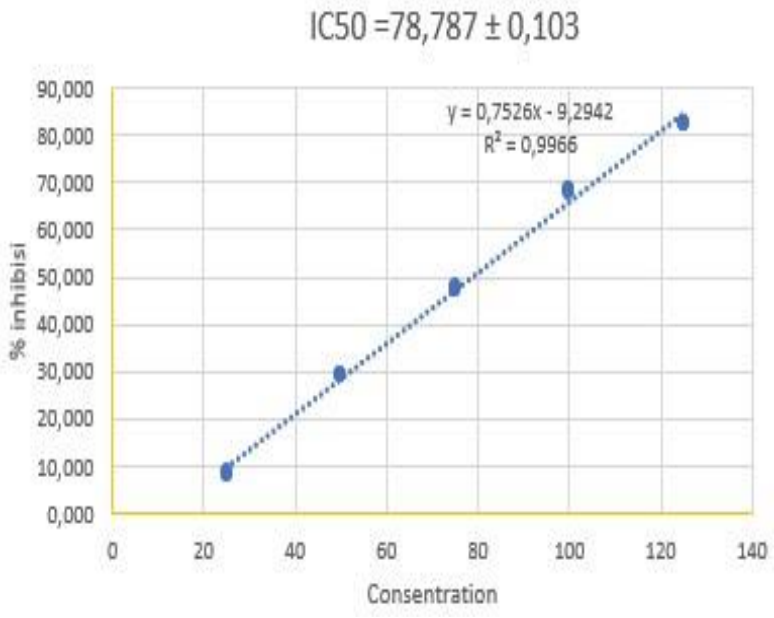

Figure 6. IC50 graph of ethanol extract of kelakai stem and leaves

Conclusion

The total flavonoid content is 76.727±0.497 ppm, and the antioxidant activity of 78.787±0.103 ppm falls into the category of strong antioxidants.

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