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# Potential Antioxidant Activity of Young and Old Breadfruit (*Artocarpus Altilis*) Leaf Extracts and Old With DPPH

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**Abstract: Potential Antioxidant Activity of Young and Old Breadfruit (*Artocarpus Altilis*) Leaf Extracts and Old With DPPH.** Bredfruit leaves (*Artocarpus atilis*) are part of a plant that is rich in active compounds that function as a source of antioxidants. In biochemical studies, breadfruit leaves are also known to have antibacterial, anti-inflammatory, and potential cancer-fighting properties. This study aims to explore the potential antioxidant activity of ethanol extract of breadfruit leaves, both young and old. The extraction process was carried out by maceration of young and old breadfruit leaf simplisia powder using 96% ethanol. The ethanol extract was given different concentrations, namely 5, 10, 20, and 40 µg/ml for young and old breadfruit leaves, while vitamin C concentrations of 0,5, 1, 2, and 4 µg/ml were used as a comparison. The method applied in this research is DPPH (*2,2-diphenyl-1-picrylhydrazyl*) method. The results showed that young breadfruit leaves had a lower IC50 value compared to old breadfruit leaves, which amounted to 32,20 µg/ml of young breadfruit leaves and 49,56 µg/ml of old breadfruit leaves, while vitamin C had an $IC_{50}$ value of 11,51 µg/ml. Based on the $IC_{50}$ value, young and old breadfruit leaves have the same category as antioxidants (very strong). In terms of antioxidant activity, young breadfruit leaves have better potential activity than old breadfruit leaves in the use as a source of natural antioxidants.

**Keywords:** Antioxidant, Young and old breadfruit leaves, dpph

***Abstrak: Potensi Aktivitas Antioksidan Ekstrak Daun Sukun (Artocarpus Altilis) Muda Dan Tua Dengan Metode DPPH***. *Daun sukun (Artocarpus atilis) merupakan bagian dari tanaman yang kaya akan senyawa aktif yang berfungsi sebagai sumber antioksidan. Dalam pembelajaran biokimia daun sukun juga dikenal memiliki sifat antibakteri, anti-inflamasi, dan potensi dalam melawan kanker. Penelitian ini bertujuan untuk mengeksplorasi potensi aktivitas antioksidan dari ekstrak etanol daun sukun baik yang muda maupun yang tua. Proses ekstraksi dilakukan dengan cara maserasi terhadap serbuk simplisia daun sukun muda dan tua menggunakan etanol 96%. Ekstrak etanol tersebut diberi konsentrasi yang berbeda, yaitu 5, 10, 20, dan 40 µg/ml untuk daun sukun muda dan tua, sementara vitamin C digunakan konsentrasi 0,5, 1, 2, dan 4 µg/ml sebagai perbanding. Metode yang diterapkan dalam penelitian ini adalah metode DPPH (2,2-diphenyl-1-picrylhydrazyl). Hasil dari penelitian menunjukkan bahwa daun sukun muda memiliki nilai $IC_{50}$ yang lebih rendah dibandingkan dengan daun sukun tua, yaitu sebesar 32,20 µg/ml daun sukun muda dan 49,56 µg/ml daun sukun tua, sementara vitamin C memiliki nilai $IC_{50}$ sebesar 11,51 µg/ml. Berdasarkan nilai $IC_{50}$ tersebut, daun sukun muda dan tua memiliki katagori yang sama sebagai antioksidan (sangat kuat).*

*Dari segi aktivitas antioksidan daun sukun muda memiliki potensi aktivitas yang lebih baik dibandingkan daun sukun tua dalam penggunaan sebagai sumber antioksidan alami.*

***Kata Kunci:*** *Antioksidan, Daun Sukun muda dan tua, dpph*

## ▪ INTRODUCTION

Breadfruit plant is one of Indonesia's natural resources. Breadfruit (*Artocarpus altilis*) is a valuable agricultural product that can contribute significantly to food diversification and food security. Known for its ability to adapt to environmental conditions, breadfruit is an ideal crop that can thrive and provide significant nutritional value, especially as a food rich in carbohydrates. Research shows breadfruit can be utilized as a main ingredient for processed products and increase their value (Supriyadi and Syafii, 2020).

The nutritious content of breadfruit is one approach to maintaining good health, which is an important component in human existence rich in nutrients and bioactive compounds, especially antioxidants. Antioxidants have an important function in protecting the body from harm caused by free radicals. The principle of antioxidants is related to the ability of compounds that can provide electrons (electron donors) that can inhibit oxidation reactions to fight free radicals in the body (Ilmiah *et al*., 2023).

In addition to the fruit being the main product in its processing, this study also explored the potential of breadfruit leaves as a health benefit for the body in its leaves which showed almost equivalent content. In fact, there are complex substances in breadfruit leaves. Research on breadfruit (*Artocarpus altilis*) leaves shows that they are a rich natural source of bioactive compounds such as flavonoids, polyphenols, and saponins, which have very strong antioxidant capabilities. A study conducted by Tanjung et al. (2021) revealed that the use of DPPH (*1,1-diphenyl-2-picrylhydrazyl*) method on ethanol extract from breadfruit leaves can significantly reduce free radical levels (Tanjung *et al*., 2021).

The content of secondary metabolite compounds found in breadfruit leaves has the potential as an antioxidant so that it has the ability to inhibit free radicals in DPPH. Free radicals are molecules that have one or more unpaired electrons, which makes them highly reactive and can damage body cells, including DNA, proteins, and cell membranes (Ilmiah *et al*., 2023). This damage can contribute to a number of degenerative diseases, including cancer, diabetes, heart disease, liver disease, stroke, and premature aging (Kurniawati and Sutoyo, 2021).

To avoid damage to cells in the body that can cause several diseases, it is necessary to consume antioxidant compounds to keep the body exposed to free radicals. Therefore, this research has an interest to use DPPH (*2,2-diphenyl-1-picrylhydrazyl*) technique to evaluate the possible antioxidant activity of young and old breadfruit (*Artocarpus altilis*) leaf extracts. This research is expected to contribute significantly to the understanding of the utilization of breadfruit leaves, both young and old, based on the activity value produced. In addition, this study also examines the potential utilization of breadfruit leaves in disease prevention as a source of natural antioxidants.

## ▪ METHOD

### Tools and Materials

Analytical balance (Fujitsu FSR-A320), beaker, measuring cup, stirring rod, knife, funnel, Whatman No. 1 filter paper, volumetric flask, blender, brown bottle, measuring pipette, micropipette, dropper, Vortex, vial, and filter (25 mesh), stopwatch, Ultraviolet-Visible (UV-Vis) spectrophotometry, *rotary evaporator* are some of the tools used in this research.

Young and old breadfruit leaves other components utilized in this study, ethanol 96% ($C_2H_5OH$), ethanol p.a ($C_5H_5OH$), DPPH powder (merck), vitamin C ($C_6H_8O_6$), aquadest ($H_2O$).

### Research Procedures

### Sample Preparation

2 kg samples of young and old breadfruit (*Artocarpus altilis*) leaves, taken in Karang Sari, Simalungun Regency, North Sumatra.

### Simplicia Powder Preparation

2 kg of young and old breadfruit (*Artocarpus altilis*) leaves were taken from the tree and then cleaned of dirt while aerated until completely clean. Furthermore, young and old leaves are cut into small pieces and then aerated at room temperature until dry and the simplisia is weighed dry weight, then blended until it becomes powder, filtered with a 25-mesh sieve.

### Preparation of Breadfruit Leaves Ethanol Extract

Extraction was carried out by immersion, carried out by means of 300 grams of simplicia powder put into a jar, then poured with 96% ethanol and kept away from light for 3 days. Then filtered with Whatman No.1 filter paper until the filtrate is obtained. The filtrate is then evaporated in a rotary evaporator until a thick extract is obtained.

### Preparation of 0.1 mM DPPH Solution

DPPH powder weighed as much as 3.9 mg and dissolved in ethanol p.a to 100.0 mL.

### Preparation of Vitamin C sample and comparison solution

Weighed extract of young and old breadfruit leaves each as much as 5 grams, then dissolve with ethanol p.a in a measuring flask of 50 ml to the limit mark, until a concentration of 10% is obtained. From levels of 10% diluted to concentrations of 5,10, 20, and 40 μg/ml.

Vitamin C as much as 1 mg dissolved with Aqua Dest up to 100 ml and obtained levels of 1%, of these levels made a series of concentrations of 0.5,1, 2, and 4 μg/ml.

### Determination of the maximum absorption wavelength of DPPH

Wavelength measurement by measuring 4.0 mL of 0.1 mM DPPH solution on a spectrophotometer with a wavelength of 400-600 nm to obtain an absorbance of 0.2-0.8.

**Determination of Operating Time of 0.1 Mm DPPH solution**

Determination of operating time is determined by reacting the standard 50 µl comparison of vitamin C plus 4.0 mL of 0.1 mM DPPH solution, homogenized with vortex for 1 minute and adsorbances measurement was performed every five minutes for 1 hour at the maximum of the obtained.

**Vitamin C Comparator Raw Activity Test**

0.1 mM DPPH solution pipetted as much as 4.0 ml and inserted into the vial, added 50 µl of each concentration of vitamin C solution then in the vortex for 1 minute until homogeneous and let stand for 30 minutes in a dark place, read the absorbance at the maximum obtained.

**Determination of antioxidant activity of young and old breadfruit (Artocarpus altilis) leaf extract**

DPPH 0.1 mM solution of 4 ml and inserted into glass bottle, added 50 µl ethanol extract of young and old breadfruit leaves with various concentrations then in the vortex for 1 minute until homogeneous and let stand for 30 minutes in a dark place, read the absorbance at the maximum.

**Technical Data Analysis**

Determination of actioxidants in the sample is determined based on the amount of inhibition of DPPH radical uptake by calculating % inhibition.

$$\% \text{ Inhibition} = \frac{control\ absorbance - sample\ absorbance}{control\ absorbance} \text{ x } 100\%$$

Description :
% Inhibition
Absorbance control : DPPH radical solution absorption
Sample adsorbent : absorption of sample solution in DPPH solution

The value of $IC_{50}$ is calculated using a linear regression equation. $IC_{50}$ is a value or number that indicates the concentration of a sample that can inhibit radical activity by 50%. Determination of $IC_{50}$ value requires standard curve equation with inhibition percent as Y axis and extract/antioxidant concentration as x axis. The lower the $IC_{50}$ value, the higher the antioxidant activity.

## ▪ RESULTS AND DISCUSSION

The results of this study obtained a thick extract of 23,6 g with a yield of 7,86% and 300 g of old breadfruit leaf simplisia powder produced 17,7 g of thick extract with a yield of 6%.

This study also shows the level of antioxidant activity of young and old breadfruit (*Artocarpus Altilis*) leaf extracts with the results of the calculation of the absorbance value of the extract in measurements using a UV-Vis spectrometer.

*Determination of Antioxidant Activity by DPPH Method Maximum Absorption Wavelength*

The purpose of the maximum wavelength (λ) is determined to ensure that the sensitivity of the sample absorbance measurement takes place optimally. Changes in absorption with changes in concentration reach the most significant value at the

maximum wavelength, resulting in the best level of analytical sensitivity (Putri *et al*., 2023). This is closely related to the Lambert-Beer law, which explains how absorbance and concentration of analytical solutions are linearly related (Zirlyvera *et al*., 2024).

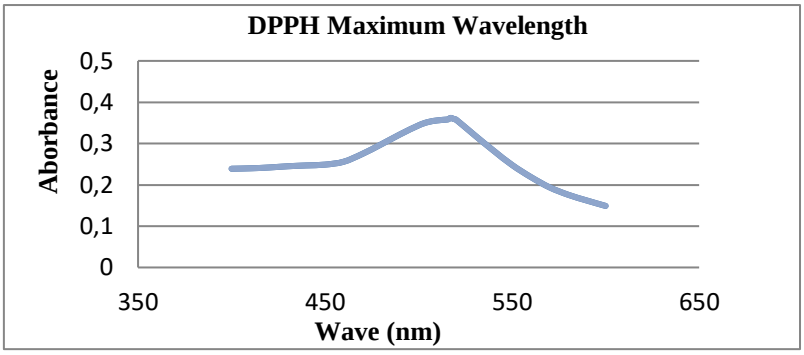


**Figure 1.** DPPH Maximum Absorption Wavelength Chart

In the scanning graph, the maximum absorption wave of DPPH shows a maximum absorption of 517 nm with an absorbance of 0,362. According to *Muliasari et al* (2023), theoretically DPPH shows maximum absorption at a wavelength of 517 nm. In this wavelength range, the DPPH mixture can give maximum absorbance which then decreases stoichiometrically when the electrons are paired. Sulistyani *et al*. (2024) also revealed that DPPH has a wavelength in the range of 515-520 nm when dissolved in alcohol solution.

**Determination of Operating Time**

The purpose of the operating time is to ensure the best time for the test solution to reduce DPPH free radicals. The operating time indicates that the reaction between the test solution and DPPH has reached perfection (Putri, 2023). The determination of this time is based on when the absorption value of the test solution against DPPH begins to show stability or when the absorbance difference between the time intervals tested becomes very small (Harahap and Ridwanto, 2024).

Measurement of operating time is done by determining the absorption value of the standard solution (positive control). DPPH with vitamin C standard solution at each wave length used at minutes 0, 5, 10, 15, 20, 25, 30, 40, 45, 50, 55, and 60 has a maximum thickness of 517 nm. The results of the operating time measurements are as shown in the graph below.

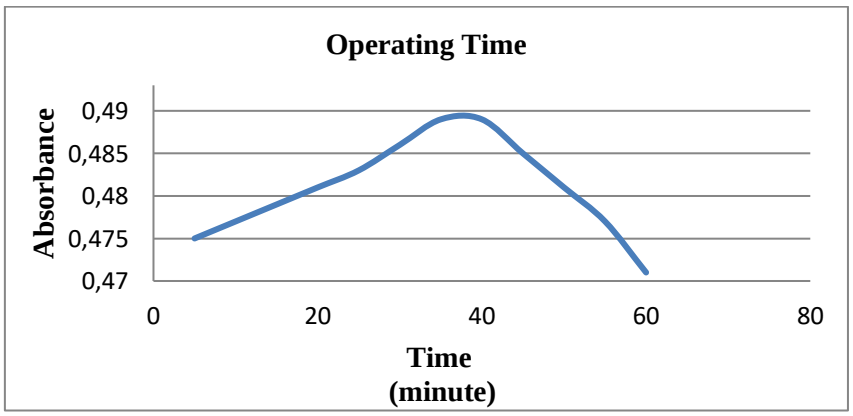


**Figure 2.** DPPH *Operating Time* Graph

Absorbance measurements were taken to determine the operating time of the solution. Comparators that have been added with 0.1 mM DPPH are stable in the time range of 30-40 minutes. The absorbance recorded was 0.489. According to the theory of Blois (1985), states that compounds with antioxidant activity that present in the comparison solution and test solution react optimally with the DPPH radicals at the 30 minute. Therefore, it can be concluded that measurements between the 30 and 40 minute will give satisfactory results.

**Antioxidant Activity with DPPH Method**

The DPPH method was chosen because it has many advantages in testing antioxidant activity. The benefits of the superior DPPH approach of the DPPH method lie in its ease of use, high sensitivity, and the ability to analyze various samples in a short time (Hidayah *et al*., 2024). Apriani and Pratiwi (2021) added that this method also offers various advantages, such as a simple, fast, and easy-to-apply analysis process, even for small amounts of samples (Apriani and Pratiwi, 2021).

The weakness of the DPPH method lies in the fact that only organic media can dissolve DPPH radicals, aqueous media cannot (Riskiana and Vifta, 2021). The opinion of Alam *et al*. (2021) which states that DPPH is also highly sensitive to light and can be damaged if not stored at the appropriate temperature (Alam *et al*., 2021).

The preliminary test is the initial stage carried out with the aim of knowing and assessing the presence of antioxidant activity in young and old breadfruit leaf extracts. This qualitative test was carried out by observing the comparison of color changes in the standard solution of young and old breadfruit leaf extracts that were being reacted using a concentration of 0.1 mM DPPH as a positive control in a combination of DPPH solution combined with conventional vitamin C solution, and as a negative control (DPPH solution only).

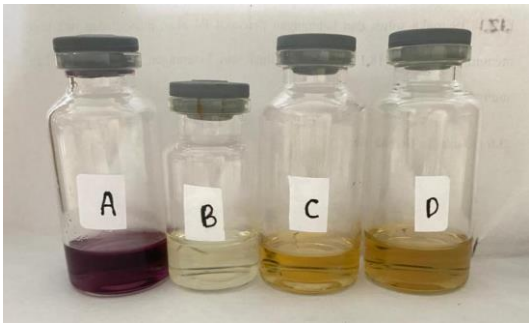


**Figure 3.** Color Change (A) DPPH Solution, (B) Vitamin C + DPPH Solution, (C) Young Breadfruit Leaf Extract Solution + DPPH, (D) Old Breadfruit Leaf Ekstrak + DPPH

Based on the test results, the antioxidant activity of extracts and vitamin C solution of young breadfruit leaves, and old breadfruit leaves showed positive results. The transition from purple to yellow colour indicates the presence of hydrogen atom donation reaction to DPPH free radicals.

Antioxidant testing using DPPH technique in this study is vitamin C as (positive control). Functioning as a secondary antioxidant, vitamin C effectively absorbs free radicals and prevents severe responses (Putri *et al*., 2023).

Vitamin C, or ascorbic acid, is a water-soluble vitamin, which is found in many types of fruit and has an important role in maintaining health. Vitamin C functions as an

antioxidant that works effectively in overcoming reactive oxygen compounds (ROS), especially free radicals (Khalish and Wulandari, 2020).

A linear regression equation describing the relationship between solution concentration and $IC_{50}$ value can be used as the (x) axis and percentage inhibition as the (y) axis. The linear regression equation obtained from the concentration versus % inhibition graph should be modified by entering the number 50 on the y-axis to determine the sample concentration. In this way, the resulting x-axis value will give the $IC_{50}$ value of the desired concentration (Hasanuddin, 2023).

The results of measuring the activity of breadfruit leaf extract as an antioxidant for young and old (*Artocarpus altilis*) and vitamin C with UV-Vis spectrophotometry are shown in table 1 below:

**Table 1.** Percent Free Radical Inhibition of Bredfruit Extract Samples and Vitamin C

| Sample | Concentration (µg/ml) | Absorbance | | | Average Absorbance | DPPH Absorbance | % Inhibition |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | | |
| Young breadfruit leaf | 5 | 0,278 | 0,287 | 0,290 | 0,285 | 0,353 | 19,26 |
| | 10 | 0,264 | 0,274 | 0,272 | 0,270 | | 23,51 |
| | 20 | 0,208 | 0,236 | 0,233 | 0,226 | | 35,97 |
| | 40 | 0,157 | 0,162 | 0,164 | 0,161 | | 54,39 |
| Old breadfruit leaf | 5 | 0,323 | 0,323 | 0,326 | 0,324 | 0,353 | 8,21 |
| | 10 | 0,310 | 0,308 | 0,309 | 0,309 | | 12,46 |
| | 20 | 0,266 | 0,264 | 0,264 | 0,264 | | 25,21 |
| | 40 | 0,210 | 0,212 | 0,211 | 0,211 | | 40,22 |
| Vit C | 0,5 | 0,341 | 0,339 | 0,340 | 0,340 | 0,353 | 3,68 |
| | 1 | 0,320 | 0,345 | 0,336 | 0,333 | | 5,66 |
| | 2 | 0,318 | 0,320 | 0,319 | 0,319 | | 9,63 |
| | 4 | 0,278 | 0,267 | 0,319 | 0,288 | | 18,41 |

Based on table 1. it can be seen that the absorption value decreases as the concentration of the sample increases, while the antioxidant activity value increases as seen from the higher % inhibition and the intensity of the purple colour which fades away indicating the reduced concentration of DPPH free radicals incorporated into the sample and comparison solution.

From table 1. The following is a graph for the $IC_{50}$ values of young and old breadfruit leaf extracts, as well as vitamin C comparison solution.

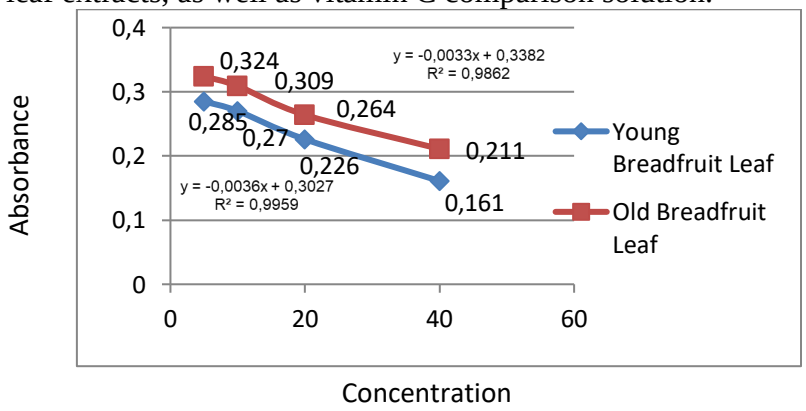

**Figure 4.** Graph of Relationship between Concentration value and Absorbance of Young and Old Breadfruit Leaf Extracts

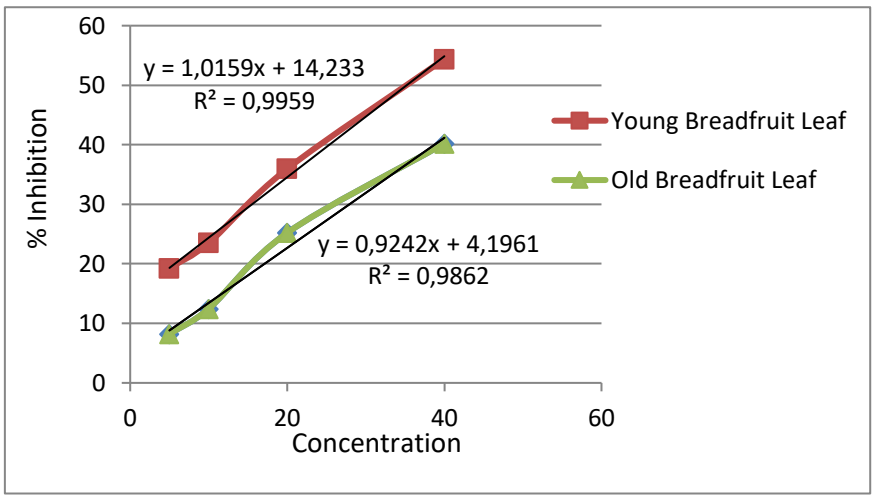


**Figure 5**. Linear Regression Equation of Antioxidant Activity of Young and Old Breadfruit Leaf Extracts

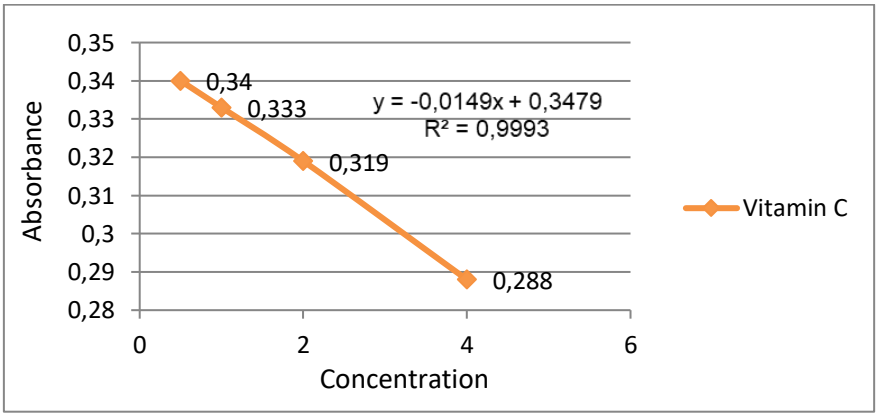


**Figure 6**. Graph of Relationship Between Concentration value and Absorbance of Vitamin C

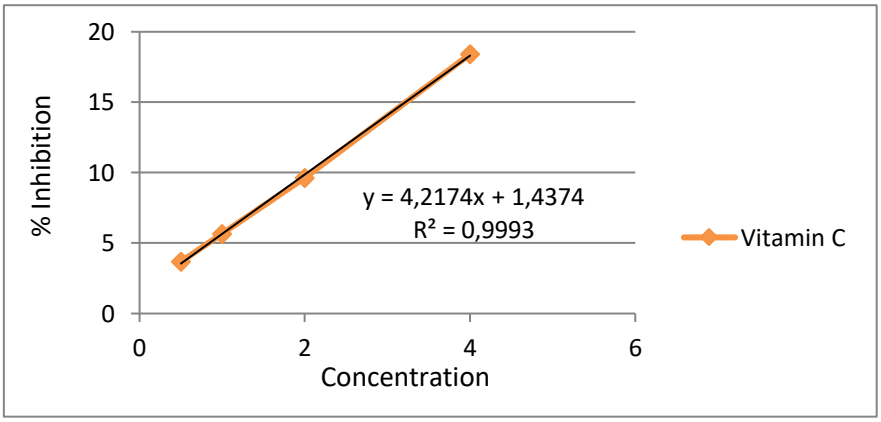


**Figure 7.** Linear Regression Equation Graph of Antioxidant Activity of Vitamin C

The decrease in the intensity of the purple colour on DPPH is an indication of this antioxidation process. The results of absorbance testing of young and old breadfruit

leaf extracts and vitamin C show that increasing concentration will affect the decrease in DPPH absorbance, seen in (Figures 4 and 7). This occurs because the higher the concentration of extracts and vitamin C solution, the more secondary metabolites in the extracts and antioxidant compounds in vitamin C, so that they can more effectively reduce DPPH radicals. As a result, the concentration of DPPH radicals decreases, which is reflected in the smaller absorbance value.

The inhibition of free radicals is indicated by the percentage of inhibition produced by young and old breadfruit leaf extracts and vitamin C solution. From Figure 5 and Figure 7 It can be seen that the higher the concentration of extract and vitamin C solution, the higher the percent inhibition value produced. So that there is a clear relationship between the concentration of vitamin C and young and old breadfruit leaf extracts and the percentage of inhibition, as indicated by the $R^2$ value which is close to 1. This indicates that the leaf extracts and vitamin C of young and old breadfruit have potential as antioxidants, with effectiveness increasing in line with the increase in concentration. Based on these results, the $IC_{50}$ value of each sample can be calculated, as shown in Table 2.

**Table 2.** Antioxidant power Level

| Sample | Linear Regression Equation | $IC_{50}$ (µg/ml) |
|---|---|---|
| Young breadfruit leaves | y = 1,0159x + 14,233<br>$R^2$ = 0,9959 | 35, 20 |
| old breadfruit leaves | y = 0,9242x + 4,1961<br>$R^2$ = 0,9862 | 49,56 |
| Vitamin C | y = 4,2174x + 1,4374<br>$R^2$ = 0,9993 | 11, 51 |

A substance is categorized if its $IC_{50}$ value is less than 50 µg/ml, it is considered a very strong antioxidant. Whereas, the $IC_{50}$ value for strong ones ranges from 50 to 100 µg/ml. For the medium category, the $IC_{50}$ value ranges from 100-150 µg/ml. Meanwhile, weak antioxidants have $IC_{50}$ values between 151-200 µg/ml. Finally, substances with $IC_{50}$ values > 200 µg/ml are included in the very weak category (Yadi and Pertiwi, 2024). The results showed that the antioxidant activity of ethanol extract of young breadfruit leaves has an $IC_{50}$ value of 35,20 µg/ml classified as (very strong). Meanwhile, the ethanol extract of old breadfruit leaves showed an $IC_{50}$ value of 49,56 µg/ml, which is included in the "very strong" category. The $IC_{50}$ value for vitamin C solution was 11,51 µg/ml, which is considered moderately strong.

Young breadfruit leaf extracts have lower $IC_{50}$ values than older breadfruit leaf extracts. This value indicates that the potential of younger breadfruit leaves actually has higher antioxidant activity than older ones because the lower $IC_{50}$ value in these samples reflects the concentration required for 50% of free radicals to be inhibited. This is consistent with the results of antioxidant activity found to be 35,20 µg/ml (Very strong) in young breadfruit leaf extract and 49,56 µg/ml (Very strong) in old breadfruit leaf extract. While in vitamin C solution the $IC_{50}$ value of 11,51 µg/ml was found to be (Very Strong).

Although theoretically the flavonoid content in young breadfruit leaves is 87,03 mg/g, which is lower than the old leaves that contain 100,68 mg/g (Kurniawati and Sutoyo, 2021). The antioxidant activity of young breadfruit leaves remains better than old leaves. The presence of secondary metabolites can be the cause, such as tannins and

saponins, which also function as antioxidant agents. Research by Wartono *et al*. (2021) showed that the more tannins, the higher the antioxidant activity. Free radicals can be combated by polyphenolic compounds that make up tannins. In addition, the presence of tannins also has a significant effect on antioxidant activity, both in precipitating proteins and in binding metals. Therefore, tannins are thought to function as biological antioxidants (Wartono *et al*., 2021).

The results of this study are also reinforced in the research of Rohiqi *et al*. (2021) which states that young leaves have a higher tannin concentration compared to old leaves. This is related to the increasing level of leaf aging, causing tannin levels to decrease. And according to Fawole and Opara (2013), the decrease in antioxidant activity can be caused by the cessation of biosynthesis of new secondary metabolites during the maturation process. In biochemical systems most of this biosynthesis occurs in the early stages of plant growth, so young leaves usually have higher levels of antioxidant activity (Fawole and Opara, 2013). Thus, this study shows that the antioxidant activity of young breadfruit leaves is higher and better used as antioxidants compared to old breadfruit leaves due to differences in the presence of secondary metabolites contained in the growth phase of leaf age.

## ▪ CONCLUSION

Breadfruit leaf extract has potential as a natural antioxidant with a quantitative level of antioxidant activity found to be 35,20 μg/ml (Very strong) in young breadfruit leaf extract and 49,56 μg/ml (Very strong) in old breadfruit leaf extract. While in vitamin C solution, the IC50 value was found to be 11,51 μg/ml (Very Strong). The ethanol extract of young and old breadfruit leaves showed an $IC_{50}$ value of < 50 μg/ml with the same category as a very strong antioxidant. However, the ethanol extract of young breadfruit leaves was found to have better antioxidant activity as a source of antioxidant compared to the old breadfruit leaves because it had a lower $IC_{50}$ antioxidant activity value compared to the old breadfruit leaves.

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