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# IN VITRO ANTIOXIDANT ACTIVITY TEST OF ETHANOL EXTRACT OF PINK CHRYSANTHEMUM LEAVES (CHRYSANTHEMUM MORIFOLIUM) USING DPPH AND FRAP METHODS

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**Abstract**
Non-communicable diseases (NCDs) are a major global health concern, with degenerative diseases accounting for >80% of premature deaths. The combination of lifestyle and environmental factors increases the production of free radicals in the body, which, when uncontrolled, cause oxidative stress that significantly contributes to various diseases. Antioxidants play a key role in stopping free radical chain reactions. Although the body has endogenous antioxidants, additional exogenous antioxidants are also needed. One source of exogenous antioxidants is pink chrysanthemum leaves, commonly known as an ornamental plant, which shows potential as an antioxidant source. This study aims to analyze phytochemical content and antioxidant activity of pink chrysanthemum leaf ethanol extract using DPPH and FRAP methods, compared with ascorbic acid. This study used laboratory experimental methods. The qualitative color tests for phytochemical screening. Antioxidant activity tested via DPPH and FRAP methods using UV-Vis spectrophotometer at 517 and 739 nm wavelengths. Results : Phytochemical screening showed positive results for steroids, tannins, phenols, flavonoids, saponins, and alkaloids. DPPH method, analyzed using regression line equation Y = 0.3547x + 45.267, showed IC50 13.344, while the ascorbic acid's 3.030. FRAP method, analyzed using regression line equation Y = 0.773x – 27.6, showed IC50 100.388, while the ascorbic acid's 18.174. The ethanol extract of pink chrysanthemum leaves contains steroids, tannins, phenols, flavonoids, saponins, and alkaloids, and possesses antioxidant activity that falls into the very strong category using the DPPH method and the strong category using the FRAP method.

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## INTRODUCTION

Non-communicable diseases (NCDs) have become a major health problem due to high morbidity and mortality rates globally (WHO, 2024). The importance of NCDs has been highlighted in the 2030 Sustainable Development Goals (SDGs) to reduce one-third of premature deaths caused by NCDs by 2030 (Wardani et al., 2024). According to the World Health Organization (WHO), degenerative diseases are the leading cause of NCD deaths, accounting for more than 80% of premature deaths with proportions including cardiovascular disease in 17.9 million people each year, cancer (9.3 million), chronic respiratory diseases (4.1 million), and diabetes (2.0 million). This condition proves that degenerative diseases contribute significantly to the burden of health development (Aryastami & Tarigan, 2017).

So far, degenerative diseases are only often associated with age factors, even though degenerative diseases are also caused by a combination of lifestyle and environmental factors. Unhealthy diet, lack of physical activity, smoking, and increased stress are the main internal triggers. Meanwhile, external exposures such as pollution, ultraviolet rays, and cigarette smoke also contribute. The interaction between these factors increases the production of free radicals in the body, which is one of the biggest contributors to the occurrence of degenerative diseases (Fandinata & Ernawati, 2020; Fatihaturahmi et al., 2023). Free radicals are unstable and highly reactive molecules because they contain unpaired electrons (Handayani et al., 2020). This high reactivity causes the molecule to attack surrounding molecules such as proteins, lipids, carbohydrates, and DNA to achieve stability. As a result, the molecule being attacked loses its electrons and turns into the free radicals themselves (Phaniendra et al., 2015). This reaction occurs continuously in the body resulting in the accumulation of free radicals that cause oxidative stress (Mulianto, 2020). Oxidative stress contributes significantly to many types of diseases (Lobo et al., 2014). Compounds that can stop the chain reaction caused by these free radicals are antioxidants (Manongko et al., 2020).

Antioxidants are compounds that have the ability to stop oxidation reactions by supplying electrons, binding free radicals and reactive molecules to prevent cell damage (Hidayah et al., 2021). Naturally, the body produces enzymatic endogenous antioxidants such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (March, 2020). However, the body's natural antioxidants alone are not enough to neutralize free radicals so they do not completely prevent cell damage. Therefore, the body needs additional antioxidants from the outside (Manongko et al., 2020). According to Cömert et al. (2020) and Sies (2019) Other antioxidants can be found in foodstuffs, including E (α-tocopherols), vitamin C (ascorbic acid), and β-carotene as well as secondary metabolic compounds such as phenolic compounds, flavonoids, and organic acids from plants (Cömert et al., 2020; Sies et al., 2017). Testing of antioxidant activity in plants is generally carried out using DPPH and FRAP methods (Nugraheni et al., 2024). As a country rich in biodiversity in the world, Indonesia has great potential in the development of natural medicines. One of Indonesia's biological natural resources is the Chrysanthemum plant (Chrysanthemum). Chrysanthemums are known as a type of flowering plant with various colors that are commonly grown as ornamental plants (Wijayanti et al., 2023). In North Sulawesi, especially Tomohon City, chrysanthemums are the most cultivated ornamental plants. In addition to its aesthetic value, chrysanthemums also have the potential as a traditional medicine, including as an antioxidant (Nayoan et al., 2023).

According to research conducted by Mahera & Firdausia, (2023) Regarding the Antioxidant Activity Test of Chrysanthemum Flower Ethanol Extract (Chrysanthemum morifolium Ramat) by DPPH Method: Water-Soluble Fraction, Ethyl Acetate, n Hexane from Lamet and Sheena Varieties Antioxidant results were obtained from chrysanthemum flower samples of lamet and sheena varieties with IC50 value category >50 - <200 ppm so that they are included in very strong to very weak antioxidant capacity.18 On the other hand, based on the research of Felicia et al (2020), flavonoid compounds were found, Alkaloids, saponins, and tannins in chrysanthemum leaves that have the potential to be antioxidants (Rawung et al., 2020). However, research on the antioxidant activity of chrysanthemum leaves has never been reported.

Although previous studies have revealed a lot of antioxidant activity from Chrysanthemums, the antioxidant potential of Pink Chrysanthemum leaves is still not widely researched. Therefore, this study has novelty in several aspects. First, this study explored Pink Chrysanthemum leaves as a source of antioxidants, in contrast to previous studies that focused more on the flower part. Second, this study uses Microwave Assisted Extraction (MAE), a modern extraction method that is more efficient than conventional methods. Third, this study tested antioxidant activity by two different methods, namely DPPH and FRAP, to obtain a more comprehensive picture of the antioxidant capacity of Pink Chrysanthemum leaves.

This study aims to analyze the content of bioactive compounds in ethanol extract of Pink Chrysanthemum leaves through phytochemical screening, test antioxidant activity using DPPH and FRAP methods, and compare the antioxidant effectiveness of ethanol extract of Pink Chrysanthemum leaves with ascorbic acid as a positive control. The results of this research are expected to provide benefits in various fields. From a scientific perspective, this research adds insight into the field of pharmacology regarding the antioxidant potential of ornamental plants. In terms of health, this research can provide an alternative source of natural antioxidants that have the potential to be developed as health supplements or raw materials for the pharmaceutical industry. From an economic perspective, this research supports the use of Chrysanthemums not only as ornamental plants, but also as value-added raw materials in the health and cosmetics industries. Thus, this research can be the basis for further development in the utilization of Pink Chrysanthemum leaves as a potential source of natural antioxidants.

## RESEARCH METHOD

This research is a laboratory experimental research. This study aims to test antioxidant activity using DPPH (2,2-diphenyl-1-pyrrylhydrazile) and FRAP (Ferric Reducing Antioxidant Power) methods in ethanol extract of Pink Chrysanthemum (Chrysanthemum morifolium) leaves. The approach used in this study is quantitative with the measurement of antioxidant activity by UV-Vis spectrophotometry to obtain numerical data related to the IC50 value of the tested extract. The population in this study is the Pink Chrysanthemum (*Chrysanthemum morifolium*) plant that grows in the Kakaskasen Tiga area, Tomohon City, North Sulawesi Province. The samples used were in the form of Pink Chrysanthemum leaves selected using the purposive sampling method, namely only healthy leaves that meet certain criteria for laboratory tests.

The data collection technique in this study consists of primary data and secondary data. Primary data were obtained through the results of testing the antioxidant activity of Pink

Chrysanthemum leaf extract using DPPH and FRAP methods. Meanwhile, secondary data is in the form of supporting data collected from literature, scientific journals, and other reliable sources related to Chrysanthemum plants and antioxidant test methods. The data analysis technique in this study was carried out quantitatively, where the data from the measurement of antioxidant activity was analyzed using a regression line equation to determine the IC50 value. Furthermore, the IC50 results obtained are classified based on the level of antioxidant strength into several categories, namely very strong, strong, moderate,

## RESULTS AND DISCUSSION

### Plant Determination

The pink chrysanthemum leaves that will be used in this study are determined by plants first. The results of plant determination carried out at the Laboratory of Taxonomy Section, Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado showed that the sample used was a pink chrysanthemum leaf plant with the Latin name (*Chrysanthemum morifolium*) from the Asteracceae family.

### Making Pink Chrysanthemum Leaf Simplisia

The making of simplicia begins with picking leaves and is directly put into a plastic bag so that the sample is not damaged (Waladati, 2023). From the picking, 1514 grams of raw materials were obtained and wet sorted to separate dirt or other foreign materials, and washed. Then it is mashed using a blender and smoothed/sifted until a fine simplicia powder of 110 grams is obtained. This is done in order to obtain a fine simplicia powder with the aim of increasing the contact surface area between the plant and the solvent, which results in a more effective extraction process of active compounds.

### Pink Chrysanthemum Leaf Extraction Results

The extraction of pink chrysanthemum leaves uses *the Microwave Assisted Extraction* (MAE) method using a 96% ethanol solvent with a ratio of 1:10 between the material and the solvent. Pink chrysanthemum leaf simplicia powder as much as 50gr is added with 96% ethanol solvent as much as 500 ml. Then it is extracted using *a microwave* with a power of 700 Watts for 6 minutes. The solution is irradiated in *the microwave* oven at regular intervals (1 minute radiation and 2 minutes turned off) to keep the temperature no more than 800C. The solution is then left at room temperature, and filtered using filter paper until a pink chrysanthemum leaf filtrate is obtained, then the filtrate is concentrated using *a rotary vacuum evaporator* and then put in an oven with a temperature of 400C until a thick extract is obtained. From the evaporation process, 6.05 viscous extracts were obtained.

$$\% \, Rendemen = \frac{berat\ ekstrak\ yang\ didapat}{berat\ simplisia\ yang\ diekstraksi} \times 100\%$$

$$= \frac{6{,}05gr}{50gr} \times 100\%$$

$$= 12{,}11\%$$

Based on the calculation above, the extract yield yielded a percentage of 12.11% which significantly met the requirements for good yield. The condition set is a yield of not less than

10%, and the results of this study have exceeded the minimum limit. The higher the yield value, the more the value of the extract produced.

**Phytochemical Screening Results**

Based on the results of phytochemical screening examinations, it is known that the ethanol extract of pink chrysanthemum leaves contains secondary metabolites, namely steroids, tannins, phenols, flavonoids, saponins, and alkaloids.

**Table 1. Phytochemical Screening Results**

| It | Compound Class | Reagents | Result | Information |
|---|---|---|---|---|
| 1. | Steroids/ Triterpenoids | H2SO4 | | |
| | | 100% glacial acetic acid | + | Appears bluish-green |
| 2. | Tannins | FeCl3 | + | Formed green color |
| 3. | Phenol | FeCl3 | + | Formed a blackish-green color |
| 4. | Flavonoids | HCL | | |
| | | Magnesium Powder | + | Formed brick red color |
| 5. | Saponins | Aquades | + | There is a stable foam |
| 6. | Alkaloids | Wagner | + | Red deposits form |
| | | Mayer | - | No precipitate formation |
| | | Dragendroff | + | Orange deposits are formed |

Information:
+ : Contains a group of compounds
- : Does not contain a group of compounds

**Results of Antioxidant Activity Test of Pink Chrysanthemum Leaf Extract with DPPH Method**

**Table 2. Results of Measurement of Absorbance Value of DPPH Solution**

| Sample | Absorbance | | | Average Absorbance |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| DPPH | 0,898 | 0,898 | 0,899 | 0,898 |

The antioxidant activity of ethanol extract of pink chrysanthemum leaves was tested using the DPPH method. The test began by making a DPPH solution and then measuring its absorption, the measurement was carried out 3 times using a Uv-Vis spectrophotomer with a wavelength of 517 nm. The sample solution is made into concentration variations of 20 ppm, 40 ppm, 60 ppm, 80 ppm, 100 ppm taken 1 ml of each sample solution mixed with 4 ml of DPPH solution in a separate test tube. Then the test solution from each concentration was pipetted as much as 2 ml and 2 ml of DPPH solution was added. Measurements were made by three repetitions using a Uv-Vis spectrophotometer with a wavelength of 517 nm.

**Table 3. Results of Measurement of Absorbance Value of Pink Chrysanthemum Leaf Extract by DPPH Method**

| Sample | Absorbance | Average | %Inhibition |
|---|---|---|---|

| | Concentration (ppm) | 1 | 2 | 3 | | |
|---|---|---|---|---|---|---|
| Pink Chrysanthemum Leaf Extract | 20 | 0,446 | 0,446 | 0,446 | 0,446 | 50,334 |
| | 40 | 0,381 | 0,381 | 0,380 | 0,381 | 57,572 |
| | 60 | 0,250 | 0,250 | 0,249 | 0,250 | 72,160 |
| | 80 | 0,232 | 0,207 | 0,203 | 0,214 | 76,169 |
| | 100 | 0,212 | 0,211 | 0,209 | 0,211 | 76,503 |

The results of the study showed a significant relationship pattern, namely along with the large concentration of the solution, there was a systematic decrease in the absorbance value and a progressive increase in the percentage of inhibition, which indicated the potential of antioxidants in reducing free radical activity.

**Ascorbic Acid Antioxidant Activity Test Results with DPPH Method**

The antioxidant activity of ethanol extract of pink chrysanthemum leaves was tested using the DPPH method. Sample solutions are made into concentration variations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm taken 1 ml of each sample solution mixed with 4 ml of DPPH solution in a separate test tube. Then the test solution from each concentration was pipetted as much as 2 ml and 2 ml of DPPH solution was added. Measurements were made by three repetitions using a Uv-Vis spectrophotometer with a wavelength of 517 nm.

**Table 4. Results of Ascorbic Acid Absorbance Value Measurement by DPPH Method**

| Sample | Concentration (ppm) | Absorbance | | | Average | %Inhibition |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| Sour Ascorbic | 2 | 0,456 | 0,455 | 0,455 | 0,455 | 49,332 |
| | 4 | 0,436 | 0,435 | 0,435 | 0,435 | 51,559 |
| | 6 | 0,383 | 0,384 | 0,383 | 0,383 | 57,350 |
| | 8 | 0,299 | 0,299 | 0,299 | 0,299 | 66,704 |
| | 10 | 0,213 | 0,215 | 0,215 | 0,214 | 76,169 |

The results showed a significant relationship pattern, namely along with the large concentration of the solution, there was a systematic decrease in the absorbance value and a progressive increase in the percentage of inhibition, which indicated the potential of antioxidants in reducing free radical activity.

**Results of Antioxidant Activity Test of Ethanol Extract of Pink Chrysanthemum Leaves with FRAP Method**

**Table 5. Results of Absorbance Value Measurement of FRAP Solution**

| Sample | Absorbance | | | Average Absorbance |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| FRAP | 0,263 | 0,263 | 0,263 | 0,263 |

The antioxidant activity of ethanol extract of pink chrysanthemum leaves was tested using the FRAP method. The test began by making a FRAP solution and then measuring its absorption, the measurement was carried out 3 times using a Uv-Vis spectrophotomer with a wavelength of 739 nm. The sample solution was made into concentration variations of 40 ppm, 50 ppm, 60 ppm, 70 ppm, 80 ppm. Measurements were made with three repetitions using a Uv-Vis spectrophotometer with a wavelength of 739 nm.

**Table 6. Results of Measurement of Absorbance Value of Pink Chrysanthemum Leaf Extract by FRAP Method**

| Sample | Concentration (ppm) | Absorbance | | | Average | %Inhibition |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| Pink Chrysanthemum Leaf Extract | 40 | 0,275 | 0,274 | 0,277 | 0,275 | 4,364 |
| | 50 | 0,279 | 0,295 | 0,288 | 0,287 | 8,362 |
| | 60 | 0,337 | 0,337 | 0,336 | 0,337 | 21,958 |
| | 70 | 0,347 | 0,348 | 0,345 | 0,347 | 24,207 |
| | 80 | 0,405 | 0,405 | 0,405 | 0,405 | 35,061 |

The results showed a significant relationship pattern, namely along with the large concentration of the solution, there was an increase in the absorbance value and inhibition percentage, which indicated the potential of antioxidants in reducing $Fe^{3+}$ ions to $Fe^{2+}$.

**Ascorbic Acid Antioxidant Activity Test Results with FRAP Method**

Antioxidant activity of ascorbic acid using the FRAP method. The sample solution was made into concentration variations of 4 ppm, 5 ppm, 6 ppm, 7 ppm, 8 ppm. Measurements were made with three repetitions using a Uv-Vis spectrophotometer with a wavelength of 739 nm.

**Table 7. Results of Measurement of Ascorbic Acid Absorbance Value by FRAP Method**

| Sample | Concentration (ppm) | Absorbance | | | Average | %Inhibition |
|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | | |
| Sour Ascorbic | 4 | 0,278 | 0,277 | 0,278 | 0,278 | 5,282 |
| | 5 | 0,287 | 0,287 | 0,288 | 0,287 | 8,470 |
| | 6 | 0,299 | 0,294 | 0,294 | 0,296 | 11,048 |
| | 7 | 0,301 | 0,302 | 0,302 | 0,302 | 12,817 |
| | 8 | 0,324 | 0,327 | 0,323 | 0,325 | 18,993 |

The results showed a significant relationship pattern, namely along with the large concentration of the solution, there was an increase in the absorbance value and inhibition percentage, which indicated the potential of antioxidants in reducing $Fe^{3+}$ ions to $Fe^{2+}$.

**IC50 Analysis Results of Pink Chrysanthemum Leaf Extract DPPH Method**

Measurement of antioxidant activity of ethanol extract of pink chrysanthemum leaves using the DPPH method produced an absorbance value. Then the percentage of inhibition was calculated by comparing the difference in absorbance before and after the addition of samples to the initial absorbance of the DPPH solution. After the percentage of inhibition of pink chrysanthemum leaf ethanol extract is known, then the IC50 value can be determined using a linear regression equation by making a relationship curve of sample concentration (µg/ml) to the percentage of inhibition (antioxidant activity) with the equation y= ax + b, sample concentration (ppm) as the axis (X) and the value of the percentage of inhibition as the axis (Y). The regression equation obtained is y = 0.3547x + 45.267 with a correlation coefficient value (R2) = 0.888.

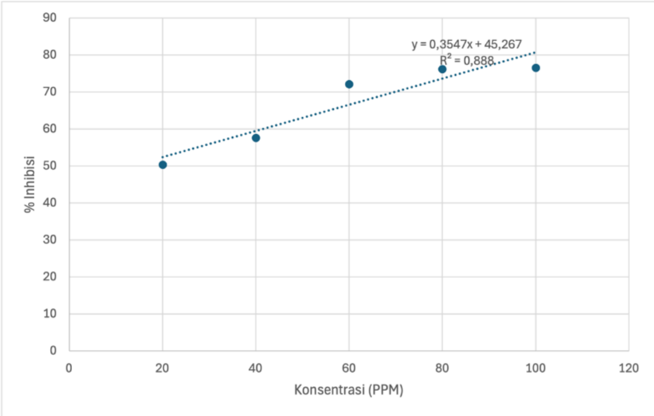


**Figure 1.** Graph of Antioxidant Activity Test Results of Pink Chrysanthemum Leaf Extract with DPPH Method

In the linear regression equation y = ax + b, a is the slope setting, b is the intercept, x is the concentration of the test solution, y is the % of antioxidant activity. The IC50 value is a number that indicates the effective concentration of pink chrysanthemum leaf ethanol extract which is able to inhibit the oxidation process by 50% of the total DPPH. So that the value of 50 is substituted for the value of y, after substituting the value of 50 for the value of y, the value of x will be obtained as the value of IC50. The values of A and B can be known from Figure 6. i.e. A = 0.3547, B = 45.267. The power of antioxidants can be categorized as very strong if they have IC50< 50 ppm values, strong in the range of 50-100 ppm, moderate between 101-250 ppm, weak in the range of 250-500 ppm, and inactive if the value is > 500 ppm. The following is the calculation of the IC50 value of pink leaf ethanol extra:

y = 0.3547x + 45.267
50 = 0.3547x + 45,267
50 – 45,267 = 0.3547x
4.733 = 0.3547x
x = 13, 344 ppm

Based on these calculations, the IC50 value of ethanol extract of pink chrysanthemum leaves is 13.344 ppm. The results show that in the ethanol extract, pink chrysanthemum leaves can reduce free radicals which are included in the very strong category.

**Results of IC50 Ascorbic Acid Analysis DPPH Method**

Measurement of antioxidant activity of ethanol extract of pink chrysanthemum leaves using the DPPH method produced an absorbance value. Then the percentage of inhibition was calculated by comparing the difference in absorbance before and after the addition of samples to the beginning of the DPPH solution. After the percentage of inhibition of pink chrysanthemum leaf ethanol extract is known, then the IC50 value can be determined using a linear regression equation by making a curve of the relationship between the sample concentration (µg/ml) and the percentage of inhibition (antioxidant activity) with the equation y = ax + b, sample concentration (ppm) as the axis (X) and the value of the percentage of inhibition as the axis (Y). The regression equation obtained is y = 3.441x + 39.577 with a correlation coefficient value (R2) = 0.9506.

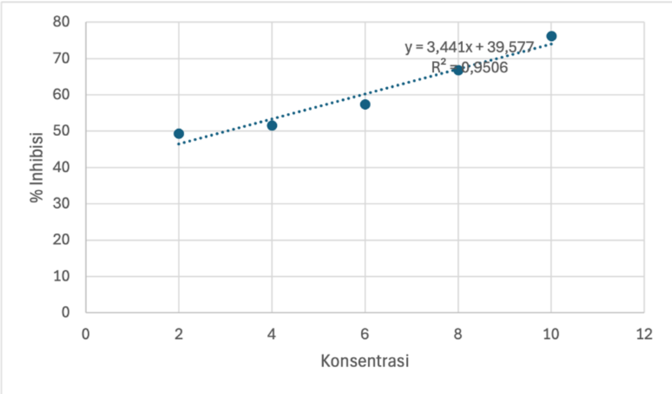


**Figure 2.** Graph of Ascorbic Acid Antioxidant Activity Test Results with DPPH Method

In the linear regression equation y = ax + b, a is the slope setting, b is the intercept, x is the concentration of the test solution, y is the % of antioxidant activity. The IC50 value is a number that indicates the effective concentration of ascorbic acid which is able to inhibit the oxidation process of 50% of the total DPPH. So that the value of 50 is substituted for the value of y, after substituting the value of 50 for the value of y, the value of x will be obtained as the value of IC50. The values of A and B can be known from Figure 7. namely A = 3.441, B = 39.577. The power of antioxidants can be categorized as very strong if they have IC50< 50 ppm values, strong in the range of 50-100 ppm, moderate between 101-250 ppm, weak in the range of 250-500 ppm, and inactive if the value is > 500 ppm. The following is the calculation of the IC50 value of ascorbic acid:

y = 3,441x + 39,577
50 = 3,441x + 39,577
50- 39,577 = 3,441x
10,423 = 3,441x
x = 3,030 ppm

Based on these calculations, the IC50 value of ascorbic acid is 3,030 ppm. These results show that ascorbic acid can reduce free radicals which are included in the very strong category.

**IC50 Analysis Results of Pink Chrysanthemum Leaf Extract FRAP Method**

Measurement of antioxidant activity of ethanol extract of pink chrysanthemum leaves using the FRAP method resulted in absorbance value. Then the percentage of inhibition was calculated by comparing the difference in absorbance before and after the addition of the sample to the beginning of the FRAP solution. After the percentage of inhibition of pink chrysanthemum leaf ethanol extract is known, then the IC50 value can be determined using a linear regression equation by making a curve of the relationship between the sample concentration (µg/ml) and the percentage of inhibition (antioxidant activity) with the equation y = ax + b, sample concentration (ppm) as the axis (X) and the value of the percentage of inhibition as the axis (Y). The regression equation obtained is y = 0.773x – 27.6 with a correlation coefficient value (R2) = 0.9603.

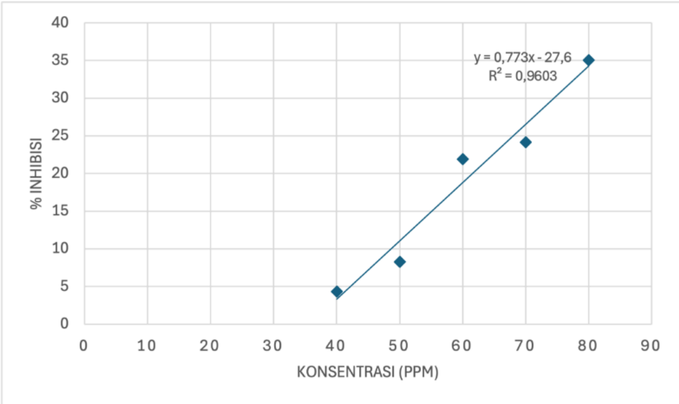


**Figure 3.** Graph of Results of Antioxidant Activity Test of Pink Chrysanthemum Leaf Extract by FRAP Method

In the linear regression equation y = ax + b, a is the slope setting, b is the intercept, x is the concentration of the test solution, y is the % of antioxidant activity. The IC50 value is a number that indicates the effective concentration of pink chrysanthemum leaf ethanol extract required to reduce 50% of $Fe^{3+}$ ions to Fe2+. So that the value of 50 is substituted for the value of y, after substituting the value of 50 for the value of y, the value of x will be obtained as the value of IC50. The values of A and B can be known from Figure 8. i.e. A = 0.773, B = - 27.6. The power of antioxidants can be categorized as very strong if they have IC50< 50 ppm values, strong in the range of 50-100 ppm, moderate between 101-250 ppm, weak in the range of 250-500 ppm, and inactive if the value is > 500 ppm. The following is the calculation of the IC50 value of ethanol extract of pink chrysanthemum leaves:

y = 0.773x + (– 27.6)
50 = 0.773x + (– 27.6)
50- (-27,6) = 0.773x
x = 100,388 ppm

Based on these calculations, the IC50 value of ethanol extract of pink chrysanthemum leaves is 100.388 ppm. The results show that in ethanol, pink chrysanthemum leaves can reduce free radicals that are included in the strong category.

**Results of IC50 Analysis of Ascorbic Acid FRAP Method**

Measurement of ascorbic acid activity using the FRAP method produced an absorbance value. Then the percentage of inhibition was calculated by comparing the difference in absorbance before and after the addition of the sample to the beginning of the FRAP solution. After the percentage of inhibition of ascorbic acid is known, then the IC50 value can be determined using a linear regression equation by making a curve of the relationship between the sample concentration (µg/ml) and the percentage of inhibition (antioxidant activity) with the equation y = ax + b, sample concentration (ppm) as the axis (X) and the value of the inhibition percentage as the axis (Y). The regression equation obtained is y = 3.1771x – 7.7404 with a correlation coefficient value (R2) = 0.9542.

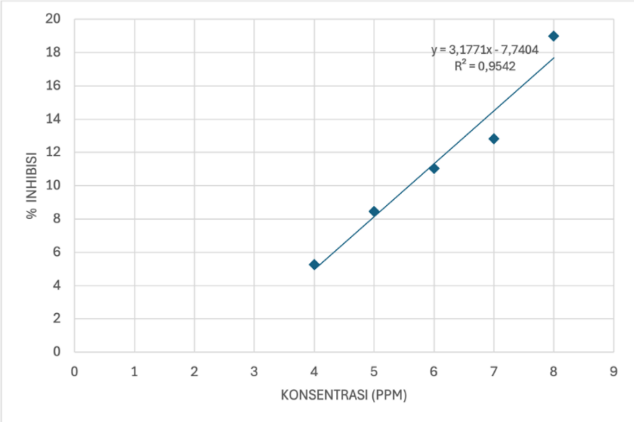


**Figure 4.** Graph of Ascorbic Acid Antioxidant Activity Test Results with FRAP Method

In the linear regression equation y = ax + b, a is the slope setting, b is the intercept, x is the concentration of the test solution, y is the % of antioxidant activity. The IC50 value is a number that indicates the effective concentration of pink chrysanthemum leaf ethanol extract required to reduce 50% of $Fe^{3+}$ ions to Fe2+. So that the value of 50 is substituted for the value of y, after substituting the value of 50 for the value of y, the value of x will be obtained as the value of IC50. The values of A and B can be known from Figure 9. i.e. A = 4.1191, B = -11.635. The power of antioxidants can be categorized as very strong if they have IC50< 50 ppm values, strong in the range of 50-100 ppm, moderate between 101-250 ppm, weak in the range of 250-500 ppm, and inactive if the value is > 500 ppm. The following is the calculation of the IC50 value of ascorbic acid:

$y = 3.1771x + (-7.7404)$
$50 = 3.1771x + (-7.7404)$
$50- (-7.7404) = 3.1771x$
$57.7404 = 3.1771x$
$x = 18,174$ ppm

Based on these calculations, the IC50 value of ascorbic acid is 18.174 ppm. These results show that ascorbic acid can reduce free radicals which are included in the very strong category.

Testing with the DPPH method showed that the ethanol extract of Pink Chrysanthemum leaves had an IC50 value of 13.344 ppm, which is categorized as a very powerful antioxidant. In comparison, the ascorbic acid used as a positive control had an IC50 of 3,030 ppm, indicating its higher effectiveness. These results indicate that Pink Chrysanthemum leaves may be a potential source of antioxidants for health applications.

Meanwhile, testing with the FRAP method produced an IC50 value of 100.388 ppm, which is included in the category of strong antioxidants. Compared to ascorbic acid which has an IC50 of 18.174 ppm, the reduction activity of Pink Chrysanthemum leaf extract to $Fe^{3+}$ ions to $Fe^{2+}$ is still lower but still has significant potential. In general, this study confirms that Pink Chrysanthemum leaves contain active compounds that play a role in antioxidant activity, so it has the potential to be further developed in the formulation of pharmaceutical products or health supplements.

## CONCLUSION

Based on the results and discussion of the research, it was concluded that the ethanol extract of Pink Chrysanthemum leaves (Chrysanthemum morifolium) showed potential as a strong source of natural antioxidants based on the results of antioxidant activity analysis using DPPH and FRAP methods. Phytochemical screening identifies the presence of bioactive compounds such as flavonoids, saponins, tannins, phenols, steroids, and alkaloids that contribute to antioxidant activity. The IC50 of the extract in the DPPH method is 13.344 μg/mL, which is included in the very strong category, while the FRAP method shows strong activity with an IC50 of 100.388 μg/mL. Compared to ascorbic acid, although the extract's IC50 value is higher, these results still prove that Pink Chrysanthemum leaves have a significant antioxidant capacity that can be harnessed for pharmaceutical and health applications.

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