Vol. 3, No. 2, 2025
DOI: 10.61975/gjset.v3i2.68

ORIGINAL ARTICLE

Antioxidant Potential of Yellow Chrysanthemum (*Chrysanthemum indicum*) Leaves: In Vitro Analysis Using DPPH and FRAP Techniques

Gabriella Therezia Ley Ley[1], Billy Johnson Kepel[2,*], Fatimawali Fatimawali[2], Aaltje Ellen Manampiring[2], Fona Dwiana Hermina Budiarso[2] and Widdhi Bodhi[2]

[1]Medical Education Study Program, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia; [2]Department of Chemistry, Faculty of Medicine, Sam Ratulangi University, Manado, Indonesia

* Correspondence: billy.kepel@unsrat.ac.id

Article History

Received
15 April 2025

Accepted
4 September 2025

Available Online
18 October 2025

Keywords

Antioxidant activity
Chrysanthemum indicum
Ethanol extract
DPPH assay
FRAP assay

Abstract

The shift from infectious diseases to non-communicable diseases (NCDs), driven by aging and unhealthy lifestyles, has led to an increase in degenerative diseases. The World Health Organization (WHO) reported that NCDs account for 74% of global deaths. An imbalance between free radicals and antioxidants contributes to the progression of these diseases, highlighting the need for research on plant-derived antioxidants. Secondary metabolites, particularly phenolic and flavonoid compounds, are abundant in *Chrysanthemum indicum* and are known for their potential antioxidant properties. This study aims to identify the secondary metabolites and evaluate the antioxidant activity of yellow chrysanthemum (*Chrysanthemum indicum*) leaves using two methods: Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH). The extraction process was performed using the reflux method with 96% ethanol as the solvent. Antioxidant activity was assessed using the DPPH and FRAP methods, with absorbance measurements conducted via UV-Vis spectrophotometry. Phytochemical screening revealed the presence of phenols, flavonoids, tannins, steroids/triterpenoids, and alkaloids in the ethanol extract of yellow chrysanthemum leaves. The antioxidant activity assay using the DPPH method indicated a value of 64.34 ppm, signifying strong antioxidant activity. In contrast, the FRAP method showed weak antioxidant activity, with an IC_{50} value of 187.13 ppm.

Introduction

The aging population and unhealthy lifestyles have shifted global health concerns from infectious diseases to non-communicable diseases (NCDs) [1]. NCDs arise when the body experiences a decline in organ function, commonly referred to as degenerative diseases [2]. Degenerative diseases can be caused by the continuous reaction of free radicals, as free radicals are unstable molecules with unpaired electrons [3]. According to WHO data in 2023, degenerative diseases account for 74% of global deaths, with 86% of premature deaths occurring before the age of 70. In Indonesia, the prevalence of diabetes mellitus reached 6.9%, contributing to 236,711 deaths in 2021. North Sulawesi ranked fourth in Indonesia, with a prevalence of 2.3% [4]. and Manado City recorded the highest prevalence in the province at 3.45%, equivalent to 6,804 cases in 2020, based on data from the Central Statistics Agency (BPS) [5].

An imbalance of free radicals in the body can lead to oxidative stress, which, in turn, may exacerbate disease progression. Yellow chrysanthemum (*Chrysanthemum indicum*) is an edible plant that is also classified as a medicinal herb. Chen et al. identified various bioactive compounds in *Chrysanthemum indicum*, including flavonoids, which exhibit antioxidant and anti-inflammatory properties. The primary phenolic compounds found in this plant include acacetin, linarin, 3,5-di-O-caffeoylquinic acid, and chlorogenic acid [6].

A study conducted by Ryu et al. demonstrated that ethanol extract from chrysanthemum leaves can mitigate obesity and its associated disorders, including insulin resistance, hepatic steatosis, and inflammation, by regulating glucose and lipid metabolism in high-fat diet (HFD)-induced obese mice [7]. The flavonoid content in yellow chrysanthemum leaves offers various health benefits, including liver protection against alcoholic liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD). Additionally, these compounds inhibit key regulatory molecules involved in inflammatory processes, such as phosphodiesterase, lipoxygenase, and cyclooxygenase (COX) [8].

Antioxidants from the phenolic hydroxyl group present in the flavonoid ring impart antioxidant properties by functioning as free radical scavengers, hydrogen donors, singlet oxygen (O_2) scavengers, and metal chelating agents. Glycosylation can reduce the antioxidant activity of phytochemical compounds. Flavonoids exert antioxidant effects due to their ability to directly scavenge reactive oxygen species (ROS) and act as ion chelators by donating hydrogen atoms. Moreover, these antioxidants also operate through a single-electron transfer mechanism, reacting with the reactive elements of ions [9]. By stabilizing ROS radicals, flavonoids render them inactive due to the strong affinity of the hydroxyl (OH) group in the phytochemical compound.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was selected due to its several advantages, including simplicity, rapidity, and applicability to various sample types, such as plant extracts. The Ferric Reducing Antioxidant Power (FRAP) method, on the other hand, offers several benefits, such as stability and reproducibility. This method provides results that are generally stable and consistent [10].

Previous studies have primarily focused on the biological effects of C. indicum extract, such as its anti-inflammatory and antimicrobial activities. However, there has been limited research comparing its antioxidant capacity using different methods. Therefore, the aim of this study is to identify the phytochemical compounds present in C. indicum leaf extract and assess its antioxidant activity using the FRAP and DPPH methods. A comparison between these two methods will provide insights into the effectiveness of the antioxidant properties, thereby determining the more sensitive method for evaluating the antioxidant potential of this plant.

Materials and Methods

Plant Determination

The yellow chrysanthemum (*Chrysanthemum indicum*) leaves used in this study were collected and taxonomically analyzed by a qualified taxonomist at the CV. BIOVINA Laboratory. The identification process involved examining the morphological characteristics of the leaf samples to accurately determine their scientific species. The plant is classified under the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Asterales, and Family Asteraceae. The genus of this plant is Chrysanthemum, and the species is *Chrysanthemum indicum*.

Materials and Equipment

The test material used was 517 grams of fresh yellow chrysanthemum (*Chrysanthemum indicum*) leaves, harvested from a chrysanthemum cultivation garden in Kakaskasen Tiga Village, Tomohon City, North Sulawesi Province. The solvent used was 96% ethanol. Other materials included yellow chrysanthemum leaf extract, glacial CH_3COOH, H_2SO_4, 1% $FeCl_3$, magnesium powder, amyl alcohol, alcohol, distilled water, oxalic acid, chloroform, Mayer's reagent, Wagner's reagent, Dragendorff's reagent, potassium ferricyanide, trichloroacetic acid, phosphate buffer, $FeCl_3$ 0,05%, DPPH , and ascorbic acid as the standard or positive control for the DPPH assay. The equipment used included containers, an analytical balance, a blender, an oven, reflux apparatus, a condenser, a hot plate, reflux flasks, a rotary evaporator, a UV-Vis spectrophotometer, cuvettes, micropipettes, a vortex mixer, and test tubes.

Sample Preparation

The leaves of yellow chrysanthemum (*Chrysanthemum indicum*) were harvested from a chrysanthemum cultivation garden located in Kakaskasen Tiga, Tomohon City, North Sulawesi Province, at an altitude of 780 meters above sea level. A total of 517 grams of yellow chrysanthemum leaves (*Chrysanthemum indicum*) were thoroughly washed, dried, and then further dried in an oven at 70°C. After drying, the leaves were blended to obtain a dry simplicia powder. The resulting powder was sieved using a 40 mesh filter. In this study, the storage of raw materials and extracts was conducted under controlled conditions to maintain the stability of active compounds. The freshly collected yellow chrysanthemum (*Chrysanthemum indicum*) leaves were stored at 4°C in the dark to prevent degradation due to light exposure and oxidation prior to the extraction process. The dried extract was then stored in a refrigerated chamber in a tightly sealed dark glass container at a temperature of 1.7–3.3°C to prevent degradation caused by oxidation, light exposure, and environmental temperature fluctuations [11].

Extraction Procedure

The extraction of yellow chrysanthemum (*Chrysanthemum indicum*) leaves was carried out using the reflux method, with a ratio of 70 g of simplicia powder to 700 ml of 96% ethanol (1:10). The extraction was performed for 2-4 hours at temperatures ranging from 60-70°C once the solvent reached its boiling point. The mixture was then filtered using a cloth filter, and the filtrate was evaporated in an oven at 40°C for two days to obtain a concentrated extract free of ethanol. The yield was measured, and the extract was stored for subsequent antioxidant activity testing. The reflux method was chosen due to its advantages in breaking down plant cell walls and facilitating the release of active compounds. According to Linghong et al., reflux extraction, compared to conventional extraction methods, offers advantages such as shorter extraction times and reduced solvent costs due to the reuse of solvents. A study by Dwi et al. that compared reflux and maceration extraction methods indicated that reflux extraction produced an extract 15 times higher than maceration when using a material-to-solvent ratio of 1:5. 96% ethanol was used as the primary solvent, which has been evaluated as effective for extracting secondary metabolites from yellow chrysanthemum leaves. The choice of 96% ethanol was based on its higher solvent strength compared to 70% ethanol, making it more efficient in extracting compounds such as antioxidants. Furthermore, its lower water content leads to a more concentrated extract, enriched with target compounds.

Phytochemical Screening

Qualitative phytochemical screening was conducted to identify secondary metabolite compounds present in the extract of yellow chrysanthemum (*Chrysanthemum indicum*) leaves. This screening included analyses of alkaloids, triterpenoids/steroids, tannins, flavonoids, saponins, and phenolics [12].

Antioxidant Activity Assay Using the DPPH Method:

The preparation of reagents involved the use of DPPH, 96% ethanol, and distilled water. A DPPH stock solution of 40 ppm was prepared by dissolving 0.004 g of DPPH in 100 ml of ethanol. Sample solutions were prepared from a 1000 ppm stock solution (0.01 g of extract dissolved in 10 ml of 96% ethanol) and diluted to final concentrations of 20, 40, 60, 80, and 100 ppm. Similarly, the standard ascorbic acid solution was prepared from a 1000 ppm stock solution (0.01 g of ascorbic acid dissolved in 10 ml of 96% ethanol), diluted to 100 ppm, and further varied to final concentrations of 2, 4, 6, 8, and 10 ppm.[13] The antioxidant activity assay was performed by mixing 2 ml of the sample solution with 2 ml of DPPH (totalling 4 ml) in five separate test tubes. The mixture was homogenized and then incubated for 30 minutes in a dark room. The absorbance of the solution was measured using a UV-Vis spectrophotometer at a wavelength of 516 nm. The blank absorbance was measured from the DPPH solution, with three repetitions using ascorbic acid as the standard, subjected to the same treatment as the samples. [14] The IC_{50} value was calculated using linear regression based on the sample concentration and the percentage of antioxidant inhibition, using the Equation 1 [15,16].

$$\%inhibition = \frac{Blank\ absorbance\ -\ Sample\ absorbance}{Blank\ absorbance} \times 100 \quad (1)$$

A curve was plotted where the x-axis represents concentration (ppm) and the y-axis represents the percentage of activity. The linear regression equation was then determined, as shown in Equation 2.

$$y = ax + b \quad (2)$$

The curve was constructed to determine the concentration required to reduce 50% of the iron ions, where a lower IC_{50} value indicates higher antioxidant activity.

Antioxidant Activity Assay Using the FRAP Method:

The preparation of reagents included the preparation of phosphate buffer, 1% potassium ferricyanide, 0.1% $FeCl_3$, 10% trichloroacetic acid, and 1% oxalic acid, each dissolved in 100 ml of distilled water in separate volumetric flasks. The sample solutions were prepared from a 1000 ppm stock solution (10 mg of extract in 96% ethanol) and then diluted to concentrations of 40, 50, 60, 70, and 80 ppm [17]. The standard solution was prepared from a 1000 ppm ascorbic acid stock solution (10 mg in 1% oxalic acid) and diluted to concentrations of 4, 5, 6, 7, and 8 ppm. For the activity assay, 1 ml of the sample was mixed with 1 ml of phosphate buffer and potassium ferricyanide, incubated for 30 minutes at 40°C, then 1 ml of TCA was added, and the mixture was centrifuged at 3000 rpm for 10 minutes. Subsequently, 1 ml of the supernatant was mixed with 1 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ and allowed to stand for 1 minute [17]. The absorbance of the sample mixture was measured using a UV-Vis spectrophotometer at a wavelength of 739 nm with three repetitions, using ascorbic acid as the standard and applying the same treatment for the FRAP method [18]. The IC_{50} value was calculated using linear regression based on the sample concentration and the percentage of antioxidant inhibition, using the formula for the percentage decrease in FRAP color:

$$\%inhibition = \frac{Sample\ absorbance\ -\ Blank\ absorbance}{Sample\ absorbance} \times 100\% \quad (3)$$

Observation and Measurement

The data generated from this study will be processed in the form of graphs and tables. Data analysis will be conducted using linear regression analysis, with calculations performed in Microsoft Excel to obtain the appropriate IC_{50} value. The data obtained from the antibacterial activity test were analyzed using the Statistical Package for the Social Sciences (SPSS) software with a One-Way ANOVA test to determine whether there were significant differences between the means of more than two groups.

Results and Discussion

Phytochemical Screening

The results of the phytochemical screening showed that the ethanol extract of yellow chrysanthemum leaves contains active compounds such as phenols, flavonoids, tannins, steroids/triterpenoids, and alkaloids, indicating a high potential for antioxidant activity. The phytochemical screening results are presented in Table 1.

Table 1. Phytochemical screening results.

No.	Examination	Reagent	Result	Description
1.	Phenols	$FeCl_3$	+	Green color formation
2.	Flavonoids	HCL Magnesium powder	+	Brick-red color formation
3.	Saponins	Aquadest	-	No stable foam formation
4.	Tannins	$FeCl_3$	+	Dark green appearance
5.	Steroids/ Triterpenoids	H_2SO_4 Glacial acetic acid 100%	+	Bluish-green appearance
6.	Alkaloids	Wagner	+	Red precipitate formation
		Mayer	-	White precipitate formation
		Dragendorf	+	Orange precipitate formation

Note : + : Contains the group of compounds; - : Does not contains the group of compounds

Antioxidant Activity Assay of Ethanol Extract from Yellow Chrysanthemum Leaves Using the DPPH Method

Table 2 presents the absorbance measurements of DPPH as a positive control, while Table 3 shows the absorbance values and inhibition percentages of the ethanol extract from yellow chrysanthemum leaves, along with ascorbic acid as a reference standard. The measurements were conducted using a UV-Vis spectrophotometer at a wavelength of 517 nm, and the absorbance values were used to determine the percentage of inhibition. The results indicate that as the concentration of the test solution increases, the absorbance value decreases, while the inhibition percentage increases, demonstrating the antioxidant activity in scavenging DPPH free radicals.

Table 2. Absorbance measurements of the DPPH solution.

Sample	Mean ± SD
DPPH	0.807 ± 0.000

Table 3. Absorbance Measurements of Yellow Chrysanthemum Leaf Extract and Ascorbic Acid Using the DPPH Method.

Sample	Concentration (ppm)	Mean ± SD	%Inhibition
Extract of Yellow Chrysanthemum	20	0.569 ± 0.001	29.53
	40	0.488 ± 0.002	39.49
	60	0.444 ± 0.010	44.98
	80	0.350 ± 0.004	56.59
	100	0.250 ± 0.002	68.98
Ascorbic Acid	2	0.552 ± 0.000	31.60
	4	0.445 ± 0.002	44.86
	6	0.247 ± 0.000	69.39
	8	0.150 ± 0.001	81.41
	10	0.113 ± 0.000	86.00

Determination of IC_{50} Value Using the DPPH Method for the Ethanol Extract of Yellow Chrysanthemum Leaves and Ascorbic Acid

The linear regression equation was obtained by plotting the concentration of the test solution against the inhibition percentage, where the y variable is set at 50 and the x variable represents the IC_{50} value, which is defined as the concentration of the solution capable of neutralizing 50% of DPPH free radicals. Figure 1 shows the results of the antioxidant activity test, with the equation obtained being (y = 0.48x + 19.116) and a correlation coefficient (R^2) of 0.984. Figure 2 shows the results of the antioxidant activity test, with the equation obtained being (y = 7.2718x + 19.005) and a correlation coefficient (R^2) of 0.9501. The IC_{50} values for the ethanol extract of yellow chrysanthemum leaves and ascorbic acid, calculated using y = ax + b, were

64.34 ppm and 4.26 ppm, respectively. These findings are supported by Maesaroh et al., who reported that varying concentrations of ascorbic acid demonstrated inhibitory effects on DPPH radicals.

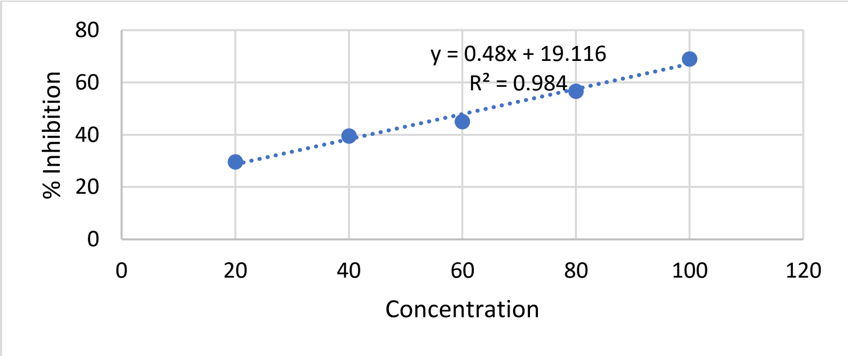

Figure 1. Graph of antioxidant activity assay of ethanol extract from yellow chrysanthemum leaves using the DPPH method.

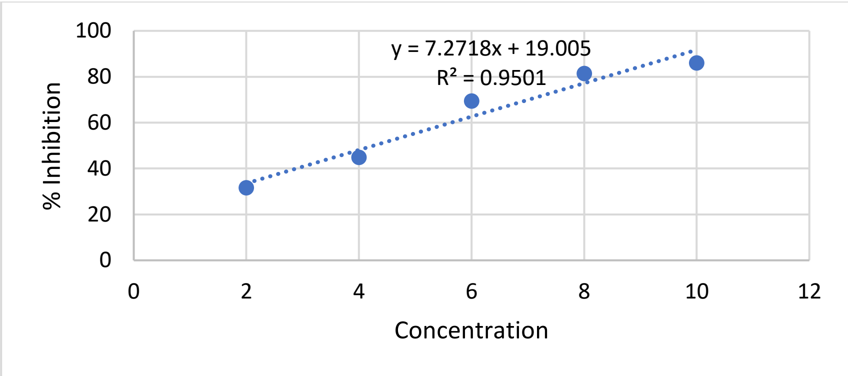

Figure 2. Graph of antioxidant activity assay for ascorbic acid using the DPPH method.

Antioxidant Activity Assay of Ethanol Extract from Yellow Chrysanthemum Leaves Using the FRAP Method

Table 5 presents the absorbance measurements of FRAP as a positive control, while Table 6 shows the absorbance values and inhibition percentages of the ethanol extract from yellow chrysanthemum leaves, along with ascorbic acid as a reference standard. The measurements were performed using a UV-Vis spectrophotometer at a wavelength of 739 nm, and absorbance values were calculated to determine the percentage of inhibition. Based on the results, it is evident that as the concentration of the test solution increases, both the absorbance value and the inhibition percentage increase.

Table 5. Absorbance measurements of the FRAP solution.

Sample	Mean ± SD
FRAP	0.265 ± 0.000

Table 6. Absorbance measurements of yellow chrysanthemum leaf extract and ascorbic acid using the FRAP method.

Sample	Concentration (ppm)	Mean ± SD	%Inhibition
Extract of Yellow Chrysanthemum	40	0.275 ± 0.001	3.64
	50	0.288 ± 0.001	7.99
	60	0.295 ± 0.002	10.07
	70	0.304 ± 0.002	12.83
	80	0.319 ± 0.001	16.84

Sample	Concentration (ppm)	Mean ± SD	%Inhibition
Ascorbic Acid	4	0.292 ± 0.002	9.35
	5	0.313 ± 0.001	15.34
	6	0.332 ± 0.001	20.18
	7	0.344 ± 0.000	22.97
	8	0.357 ± 0.001	25.84

Determination of IC_{50} Value Using the FRAP Method for Ethanol Extract of Yellow Chrysanthemum Leaves and Ascorbic Acid

The linear regression equation was obtained by plotting the concentration of the test solution against the inhibition percentage using Microsoft Excel, where the y variable is set at 50 and the x variable represents the IC_{50} value, which is defined as the concentration of the solution capable of neutralizing 50% of FRAP free radicals. Figure 3 shows the results of the antioxidant activity test, with the equation obtained being (y = 0.3125x – 8.479), and a correlation coefficient (R^2) of 0.9867. Figure 4 shows the results of the antioxidant activity test, with the equation obtained being (y = 4.0609x – 5.631), and a correlation coefficient (R^2) of 0.9695. The IC_{50} values for the ethanol extract of yellow chrysanthemum leaves and ascorbic acid, calculated using y = ax + b, were 187.13 ppm and 13.70 ppm, respectively. These findings are consistent with the study by Susanto et al., which stated that ascorbic acid possesses very strong antioxidant properties due to its molecular structure containing four hydroxyl groups, allowing it to effectively capture and neutralize free radicals.

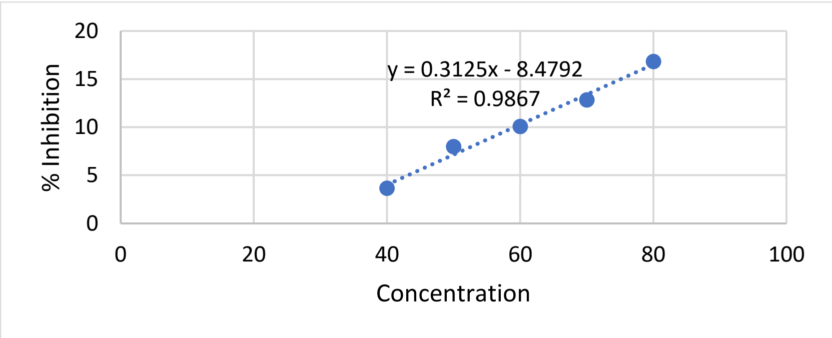

Figure 3. Graph of antioxidant activity assay of ethanol extract from yellow chrysanthemum leaves using the FRAP method.

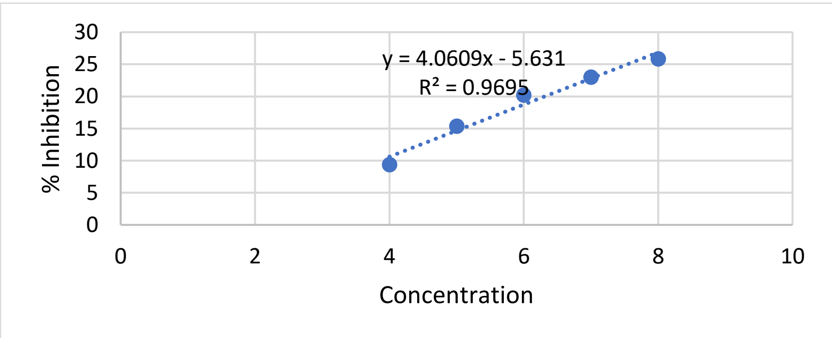

Figure 4. Graph of antioxidant activity assay for ascorbic acid using the FRAP method.

The concentration range of the extract and ascorbic acid in the DPPH and FRAP assays was determined based on preliminary testing results to ensure optimal response and linearity in absorbance measurements. A lower concentration of ascorbic acid was selected due to its high antioxidant activity, while a higher concentration of the extract was chosen to ensure that antioxidant activity could be detected significantly in both testing methods [19]. Ascorbic acid, commonly known as vitamin C, is a water-soluble compound with antioxidant properties.

Structurally, ascorbic acid contains four hydroxyl (OH) groups, which can donate additional hydrogen atoms to neutralize free radicals. Therefore, ascorbic acid was selected as the standard reference solution for the yellow chrysanthemum leaf extract. In addition to ascorbic acid, other reference compounds frequently used in antioxidant activity analysis include trolox, butylated hydroxytoluene (BHT), and quercetin. These compounds can be utilized in future studies for broader comparative analysis, providing a more comprehensive understanding of the antioxidant capacity of the tested extracts [20].

In this study, the evaluation of the antioxidant activity of yellow chrysanthemum leaf extract was performed using two different approaches, namely DPPH and FRAP. Both methods aim to assess antioxidant capacity; however, each operates through a distinct mechanism. The DPPH antioxidant activity assay involves the reaction of antioxidant compounds with DPPH radicals through a hydrogen atom donation mechanism to obtain an electron pair, whereas the FRAP antioxidant activity assay is based on the principle that antioxidants can reduce ferric (Fe^{3+}) complexes, which are yellow, to ferrous (Fe^{2+}) complexes, which are blue-green, through an electron donation process from the antioxidant compounds.

The IC_{50} value determined by the DPPH method is 64.34 ppm, which falls into the strong antioxidant category, while the FRAP method yields an IC_{50} value of 187.13 ppm, categorizing it as weak. The IC_{50} value is determined based on the concentration of the solution required to neutralize 50% of free radicals. An IC_{50} value of less than 50 ppm indicates a very strong antioxidant, 50-100 ppm indicates a strong antioxidant, 100-150 ppm indicates a moderate antioxidant, and 150-200 ppm indicates a weak antioxidant [20]. An IC_{50} value above 200 ppm suggests a very weak antioxidant. This is in line with the study by Kiki et al., which compared DPPH, FRAP, and FIC antioxidant activity assays, indicating that the DPPH method was the most effective and efficient among the three methods used, with IC_{50} values. This was also supported by Dolongtelide et al., where the ethanol extract of yellow chrysanthemum flowers (*Chrysanthemum indicum* L.) demonstrated "very strong" antioxidant activity, with the IC_{50} value indicating that both the leaves and flowers of yellow chrysanthemum possess strong antioxidant activity [21].

The difference in principles between these methods can lead to variations in the results obtained, with antioxidant activity being higher in this study using the DPPH method. This may be attributed to the high content of phenolic and flavonoid compounds in the extract, which act as natural antioxidants. These compounds contain hydroxyl (-OH) groups that can donate hydrogen atoms or electrons to DPPH radicals, thereby reducing the number of free radicals in the test system. The FRAP (Ferric Reducing Antioxidant Power) method measures the ability of an antioxidant to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). If an extract contains a significant amount of flavonoid compounds with a weaker reduction potential, the antioxidant activity measured by the FRAP method will tend to be lower compared to the DPPH method [22]. This finding is consistent with the research by Dewi et al., which showed that the DPPH method is more effective in measuring the antioxidant activity of ethanol extract from yellow chrysanthemum leaves.

The variation in the results obtained may also be due to the limitations of the methodologies used. The DPPH method has an advantage in detecting a wide range of antioxidants, including flavonoids and polyphenols, while the FRAP method is more selective for compounds with high reduction potential. Furthermore, other factors such as the acidity of the solution, the stability of DPPH radicals, and the reaction conditions also influence the sensitivity of each method. For instance, DPPH radicals tend to be more stable at low to neutral pH, whereas the FRAP method requires an acidic environment for the optimal reduction of Fe^{3+}. Additionally, the FRAP method has a drawback in that its reagents tend to be less stable and must be freshly prepared and used immediately [23].

Based on the phytochemical characteristics of the yellow chrysanthemum leaf extract, it contains dominant compounds such as flavonoids, with a greater free radical scavenging activity compared to its iron ion reduction capacity. Therefore, the antioxidant activity measured by the DPPH method was higher than that measured by the FRAP method. These findings are consistent with the study by Syamsu et al., which demonstrated that flavonoids play a key role as antioxidants by donating electrons (hydrogen) to free radicals to prevent damage [24].

The antioxidant activity in the ethanol extract of yellow chrysanthemum leaves is attributed to several secondary metabolites contained within it. Among these, flavonoids play a crucial role as antioxidants. These compounds exhibit significant antioxidant protection through four main mechanisms: First, they scavenge free radicals by donating hydrogen atoms from hydroxyl groups, thereby stopping the chain reaction of radical formation that damages cells. Second, they bind to harmful metal ions such as iron and copper, which could potentially trigger oxidative damage. Third, they inhibit enzymes involved in the formation of reactive oxygen species (ROS), such as xanthine oxidase, contributing to the reduction of oxidative stress at the cellular level. Fourth, they enhance the expression of antioxidant defense genes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, thereby strengthening the body's defense system against oxidative damage [25]. Additionally, phenolic compounds possess antioxidant capabilities that can increase antioxidant enzyme activity and protect cellular structures from oxidative damage .

The extract from yellow chrysanthemum (*Chrysanthemum indicum*) leaves contains various bioactive compounds, primarily flavonoids, phenols, and tannins, which exhibit strong antioxidant activity. Flavonoid compounds are believed to have antidiabetic properties by inhibiting protein tyrosine phosphatase 1B (PTP1B) and facilitating the translocation of glucose transporter 4 (GLUT4) in skeletal muscle, as well as enhancing the expression of insulin receptor substrate 1 (IRS-1), leading to increased insulin secretion [26]. A study by Ruo et al. reported that a flavonoid-rich diet significantly reduces mortality associated with cardiovascular disease (CVD), which is directly linked to various stages of atherosclerosis progression [27].

Conclusions

The ethanolic extract of yellow chrysanthemum leaves (*Chrysanthemum indicum*) contains active compounds, including phenols, flavonoids, tannins, steroids/triterpenoids, and alkaloids. The antioxidant activity of the ethanolic extract, evaluated using the DPPH method, was classified as strong. In contrast, its antioxidant activity assessed by the FRAP method was considered weak. In this study, the higher antioxidant capacity observed in the DPPH assay is attributed to several factors, particularly the presence of secondary metabolites that function as natural antioxidants. These compounds contain hydroxyl (-OH) groups that can donate hydrogen atoms or electrons to DPPH radicals, thereby reducing the number of free radicals in the test system. The strong antioxidant activity of the ethanolic extract of purple chrysanthemum (*Chrysanthemum indicum*) leaves holds potential for mitigating degenerative diseases such as diabetes mellitus and preventing atherosclerosis. However, this study solely determined antioxidant capacity using two widely employed methods. Future research should explore more specific antioxidant evaluation techniques, as well as investigate the detailed composition of secondary metabolites in chrysanthemum leaves, including their toxicity and biomolecular roles in disease progression.

Funding: This study does not receive external funding.

Ethical Clearance: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: All the authors declare that there are no conflicts of interest.

References

[1] Widyaningsih A. Menurunkan Tingkat Stres dan Penyakit Degeneratif dengan Pendekatan Focus Grup Discussion di PT Kayu Lapis Indonesia. *Indonesian Journal of Community Empowerment (IJCE)* 2019;1.

[2] Sheikh AM, Yano S, Tabassum S, Nagai A. The Role of the Vascular System in Degenerative Diseases: Mechanisms and Implications. *International Journal of Molecular Sciences* 2024;25. https://doi.org/10.3390/ijms25042169.

[3] Irianti TTatang. Antioksidan dan kesehatan. Gadjah Mada University Press; 2021.

[4] Mokalu FL, Natalia G, Masi M, Sirait I. Pengetahuan Remaja di Kota Manado Tentang Penyakit Degeneratif. *Mapalus Nursing Science Journal* 2023;1:12–21.

[5] Rakainsa K sanda, Widhihastuti E, Efrilianda AD. Deteksi Dini dan Edukasi Untuk Pencegaham Penyakit Degeneratif Serta Pengembangan Produk Herbal Instan Pada Masyarakat Desa Gogik, Ungaran Barat. *Abdimas Siliwangi* 2023;06:403–16.

[6] Hartanto R, Fitri SR, Kawiji K, Prabawa S, Sigit B, Yudhistira B. Physical, Chemical, and Sensory Analysis of White Chrysanthemum (*Chrysanthemum morifolium* Ramat.) Tea with Cabinet Drying. *Agrointek: Journal of Agricultural Industry Technology* 2021;15:1011–25. https://doi.org/10.21107/agrointek.v15i4.10531.

[7] Ryu R, Kwon E-Y, Choi J-Y, Shon JC, Liu K-H, Choi M-S. Chrysanthemum Leaf Ethanol Extract Prevents Obesity and Metabolic Disease in Diet-Induced Obese Mice via Lipid Mobilization in White Adipose Tissue. *Nutrients* 2019;11:1347. https://doi.org/10.3390/nu11061347.

[8] Mohamad NV, Zahari CNMC. Biological activities of Chrysanthemum morifolium and Chrysanthemum indicum: Molecular prospective. *Journal of Applied Pharmaceutical Science* 2024. https://doi.org/10.7324/JAPS.2024.171645.

[9] Sharma R, Majee C, Mazumder R, Padhi S, Khan F, Pal RS. Insight into the Regulation of Nrf2/Keap 1 Pathway by Flavonoids as an Approach for Treatment of Liver Diseases: A Review. *Indian Journal of Pharmaceutical Education and Research* 2024;58:S40–57. https://doi.org/10.5530/ijper.58.1s.4.

[10] Setiabudi A, Pringgenies D, Ridlo A. DPPH Radical Scavenging Activity and Reducing Power of Gracilaria verrucosa Extract. *JRST (Jurnal Riset Sains Dan Teknologi)* 2020;4:47. https://doi.org/10.30595/jrst.v4i2.5761.

[11] Naufal A, Kusdiyantini E, Raharjo B. Identifikasi Jenis Pigmen Dan Uji Potensi Antioksidan Ekstrak Pigmen Bakteri Serratia marcescens Hasil Isolasi Dari Sedimen Sumber Air Panas Gedong Songo. *Bioma : Berkala Ilmiah Biologi* 2018;19:95. https://doi.org/10.14710/bioma.19.2.95-103.

[12] Manongko PS, Sangi MS, Momuat LI. Uji Senyawa Fitokimia dan Aktivitas Antioksidan Tanaman Patah Tulang (Euphorbia tirucalli L.). *Jurnal MIPA* 2020;9:64. https://doi.org/10.35799/jmuo.9.2.2020.28725.

[13] Hartaman NR, Abidin Z, Dahlia AA. Aktivitas Antioksidan Ekstrak Etanol Umbi Porang (*Amorphophallus oncophyllus*) Dengan Metode Ekstraksi Ultrasonik. *Makassar Natural Product Journal* 2023;1:2023–155.

[14] Esati NK, La EOJ, Lestari GAD. Uji Aktivitas Antioksidan Ekstrak Etanol Daun Rosemary (Rosemarinus officinalis L.) dengan Metode DPPH dan FRAP serta Pengaplikasiannya sebagai Zat Aktif dalam Losion: Antioxidant Activity Test of Rosemary Leaves (Rosemarinus officinalis L.). *Sains Dan Kesehatan* 2022;4:363–369.

[15] Septian M, Wahyuni FD, Nora A. Uji Aktivitas Antioksidan dengan Metode DPPH dan Identifikasi Golongan Metabolit Sekunder pada Daging Ubi Jalar dari Berbagai Daerah di Indonesia. *Jurnal Kimia Dan Pendidikan Kimia* 2022;4:185–96. https://doi.org/10.20414/spin.v4i2.5734.

[16] Dolongtelide JI, Fatimawali F, Tallei TE, Suoth EJ, Simbala HEI, Antasionasti I, et al. In Vitro Antioxidant Activity of Chrysanthemum indicum Flowers Extract and Its Fraction. *Malacca Pharmaceutics* 2023;1:43–7. https://doi.org/10.60084/mp.v1i2.26.

[17] Setiabudi A, Pringgenies D, Ridlo A. Aktivitas Penangkapan Radikal Bebas DPPH dan Daya Reduksi Ekstrak Gracilaria verrucosa. *JRST (Jurnal Riset Sains Dan Teknologi)* 2020;4:47. https://doi.org/10.30595/jrst.v4i2.5761.

[18] Phan DTA, Bui TH, Thi Doan TQ, van Nguyen N, Ly TH. Inhibition of melanosis in whiteleg shrimp (*Litopenaeus vannamei*) during refrigerated storage using extracts of different avocado (Persea americana Mill.) by-products. *Preventive Nutrition and Food Science* 2021;26:209–18. https://doi.org/10.3746/pnf.2021.26.2.209.

[19] Julizan N, Maemunah S, Dwiyanti D, Al Anshori J. Validation of Antioxidant Activity Determination by DPPH Method. 2019;1.

[20] Fatmawati IS, Ode Mulyana W. Antioxidant Activity Test of Ethyl Acetate Extract of Bilimbi (*Averrhoa bilimbi* L.) Leaves Using the DPPH Method. *Jurnal Kimia Dan Pendidikan Kimia* 2023;12.

[21] Maesaroh K, Kurnia D, Al Anshori J. Comparison of Antioxidant Activity Assay Methods: DPPH, FRAP, and FIC Against Ascorbic Acid, Gallic Acid, and Quercetin. *Chimica et Natura Acta* 2018;6:93. https://doi.org/10.24198/cna.v6.n2.19049.

[22] Maryam S, Baits M, Nadia A. Measurement of Antioxidant Activity of Ethanolic Extract of Moringa Leaves (Moringa oleifera Lam.) Using the FRAP (Ferric Reducing Antioxidant Power) Method. *Indonesian Journal of Phytopharmaca* 2019;2.

[23] Aryanti Ri, Perdana Fa. Study Of Antioxidan Activity Testing Methods Of Green Tea (Camellia sinensis (L.) Kuntze). *Jurnal Surya Medika* 2021;7.

[24] Nur S, Sami FJ, Awaluddin A, Afsari MIA. Correlation Between Total Flavonoid and Phenolic Content of Extract and Fractions of White Teak Leaves (*Gmelina arborea* Roxb.) with Antioxidant Activity. *Galenika Journal of Pharmacy (e-Journal)* 2019;5:33–42. https://doi.org/10.22487/j24428744.2019.v5.i1.12034.

[25] Qomaliyah EN, Indriani N, Rohma A, Islamiyati R. Phytochemical Screening, Total Flavonoid Content, and Antioxidant Activity of Kalanchoe pinnata Linn. *Curr Biochem 2023* 2023;10.

[26] Dwi Jayanti AS, Sitasiwi AJ, Isdadiyanto S. Effect of Purslane (Portulaca oleracea L.) Ethanol Extract in Alloxan-induced Hyperglycemic Rats (*Rattus novergicus* L.) Blood Glucose Levels. *Jurnal Sain Veteriner* 2022;40:237. https://doi.org/10.22146/jsv.61937.

[27] Li R-L, Wang L-Y, Liu S, Duan H-X, Zhang Q, Zhang T, et al. Natural Flavonoids Derived From Fruits Are Potential Agents Against Atherosclerosis. *Frontiers in Nutrition* 2022;9. https://doi.org/10.3389/fnut.2022.862277.