

**RESEARCH ARTICLE**

**EVALUATION OF ANTIOXIDANT CAPACITY AND TOXICITY OF  
CHRYSANTHEMUM FLOWER (*Chrysanthemum morifolium*) EXTRACT  
(EVALUASI KAPASITAS ANTIOKSIDAN DAN TOKSISITAS EKSTRAK BUNGA  
KRISAN (*Chrysanthemum morifolium*))**

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**ABSTRACT**

The increasing trend of air pollution has worsened the air quality and deteriorated human health. This situation subsequently causes the accumulation of free radicals, leading to oxidative stress, and eventually the emergence of various diseases such as respiratory and degenerative disorders. Indonesia is home to a wide variety of plants that may possess medicinal beneficial and efficacy. Chrysanthemum (*Chrysanthemum morifolium*) is one of the most commonly found flowers and is known to contain phytochemicals. Therefore, this research aimed to determine the antioxidant activity and toxicity level of *Chrysanthemum morifolium* flower extract. In brief, chrysanthemum flowers were dried and extracted, screened for phytochemical content, and tested for antioxidant activity using the Ferric Reducing Antioxidant Power (FRAP) methods. Toxicity assay was determined using the Brine Shrimp Lethality Test. The results showed that the chrysanthemum extract contained several phytochemicals, including alkaloids, flavonoids, terpenoids, betacyanins, and phenolics. The antioxidant activity test yielded an  $IC_{50}$  value of 11.13  $\mu$ g/mL, indicating a very strong antioxidant activity. Meanwhile the toxicity test showed an  $LC_{50}$  value of 15.49  $\mu$ g/mL. In conclusion, chrysanthemum flowers exhibit very strong antioxidant activity and have potential as an antimitotic agent.

**Keywords:** antioxidant, chrysanthemum flower, phytochemicals, toxicity assay

**ABSTRAK**

*Polusi udara yang semakin meningkat, membuat kualitas udara menjadi sangat buruk dan mempengaruhi kondisi kesehatan manusia, yaitu menyebabkan peningkatan radikal bebas yang berpotensi menimbulkan stress oksidatif dan dapat berakibat pada munculnya berbagai penyakit seperti gangguan pernafasan dan penyakit degeneratif. Indonesia kaya akan berbagai ragam tanaman yang memiliki manfaat serta berkhasiat sebagai obat, salah satunya adalah bunga krisan yang banyak ditemui secara umum dan telah diketahui memiliki kandungan berbagai fitokimia. Sehingga tujuan dari penelitian ini adalah untuk mengetahui*

*kadar aktivitas antioksidan serta kadar toksisitas dari ekstrak bunga krisan. Secara singkat, metode yang digunakan dalam penelitian ini adalah bunga krisan dikeringkan dan diekstraksi, dilakukan skrining fitokimia, dilanjutkan dengan uji aktivitas antioksidan dilakukan dengan menggunakan metode Ferric Reducing Antioxidant Power (FRAP), serta uji toksisitas yang dilakukan dengan menggunakan metode Brine Shrimp Lethality Test (BSLT). Hasil yang didapatkan adalah terdapat berbagai fitokimia pada bunga krisan seperti alkaloid, flavonoid, terpenoid, betasanin serta fenolik, hasil uji kapasitas antioksidan dengan metode FRAP didapatkan  $IC_{50}$  sebesar 11,13  $\mu\text{g}/\text{mL}$  dimana menunjukkan aktivitas antioksidan yang tergolong sangat kuat. Sedangkan untuk hasil uji toksisitas didapatkan hasil  $LC_{50}$  sebesar 15,49  $\mu\text{g}/\text{mL}$ . Melalui keseluruhan hasil tersebut dapat disimpulkan bahwa bunga krisan memiliki aktivitas antioksidan yang sangat kuat serta memiliki potensi sebagai antimitosis.*

*Kata kunci:* antioksidan, bunga krisan, fitokimia, uji toksisitas

## INTRODUCTION

Poor air quality negatively impacts public health and may elevate free radical levels, leading to increased oxidative stress. Although free radicals are naturally generated as part of normal metabolic processes, excessive amounts may disrupt physiological systems and damage structural macromolecules such as proteins, lipids, and DNA. This damage contributes to the development of various diseases, including respiratory disorders, cardiovascular disease, and other degenerative conditions. To counteract these harmful effects, the intake of antioxidant has become a key strategy. In particular, natural antioxidants are gaining importance in the food industry, where the incorporation of bioactive compounds not only improves product quality but also enhances health-promoting properties.<sup>1</sup>

Numerous plants used in traditional medicine contain secondary metabolites with antioxidant activity.<sup>2</sup> Indonesia is

home to more than 30,000 plant species, with approximately 9,000 recognized for their medicinal properties. *Chrysanthemum morifolium*, a widely cultivated ornamental flower, is particularly rich in phytochemicals.<sup>3,4</sup> Its beneficial effects are largely attributed to flavonoids and phenolic acids.<sup>5</sup> Flavonoids, one of the major classes of secondary metabolites, exhibit potent antioxidant activity and are commonly found in plants. Other notable antioxidant compounds include carotenoids, ascorbic acid, tocopherols, and various phenolics. Previous studies have shown that the antioxidant potential of chrysanthemum flowers varies with their geographical origin.<sup>6</sup> Moreover, the phenolic constituents of natural products are generally associated with strong antioxidant activity.<sup>7</sup> Despite Sukabumi, West Java, being a major chrysanthemum-producing region, the antioxidant capacity and toxicity of its chrysanthemum flowers have not been well

characterized. Therefore, this study aimed to evaluate the antioxidant capacity and toxicity of chrysanthemum flowers from Sukabumi as a potential source of natural antioxidants.

## MATERIALS AND METHODS

This study was an in vitro experimental and bioassay-based investigation, conducted at the Biochemistry and Molecular Biology Laboratory, Faculty of Medicine, Tarumanagara University, West Jakarta, from January to April 2024. The samples used were purple chrysanthemum (*Chrysanthemum morifolium*) originating from Sukabumi, West Java, which were obtained from the Rawa Belong flower market in West Jakarta.

The materials used in this study consisted of chrysanthemum flower extract, distilled water (aquadest), methanol, ethanol, ferric chloride, sodium hydroxide, concentrated sulfuric acid solution, chloroform, hydrochloric acid, Folin-Ciocalteu reagent, saturated sodium carbonate, sodium tungstate, sodium molybdate, ABTS reagent, glacial acetic acid, ammonium solution, dimethyl sulfoxide (DMSO), seawater, and *Artemia salina* larvae.

### Preparation of Chrysanthemum Flower Extract

Fresh chrysanthemum flowers were air-dried at room temperature for 3–5 days. Once completely dried, the flowers were ground into simplicia powder, which was macerated with methanol and subsequently subjected to percolation to obtain the extract. The resulting extract was then concentrated using a rotary evaporator to remove the methanol, yielding a thicker crude extract.

### Phytochemical Screening and Total Phenolic Content Assay

Several phytochemical tests were performed on the chrysanthemum flower extract, including tests for alkaloids,<sup>8–10</sup> flavonoids,<sup>11</sup> terpenoids,<sup>12</sup> anthocyanins-betacyanins,<sup>13</sup> and phenolic compounds.<sup>14,15</sup>

For the alkaloid test, 1 mL of chrysanthemum flower extract was mixed with 1 mL of 1% HCl, then heated, filtered, and divided equally into two test tubes. Two drops of Mayer's reagent were added to the first tube, and two drops of Wagner's reagent to the second. The sample was considered positive for alkaloids if a cloudy white precipitate formed in both tubes.

For the flavonoid test, 3 mL of extract was mixed with 4 mL of 1 N NaOH solution in a test tube. A positive reaction was indicated by the formation of a dark yellow color.

For the terpenoid test, 1 mL of extract was combined with 2 mL of chloroform, mixed, and dried. Concentrated

sulfuric acid was then carefully added. The sample was considered positive for terpenoids if a pink coloration appeared at the interface.

For the anthocyanin–betacyanin test, 1 mL of NaOH solution was added to chrysanthemum flower extract and heated at 100°C for 5 minutes. The appearance of a greenish–blue coloration indicated the presence of anthocyanins, whereas a yellow coloration indicated betacyanins.

For the phenolic test, 1 mL of extract was mixed with 2 mL of distilled water, followed by the addition of 0.5 mL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and 0.5 mL of Folin’s reagent. The presence of phenolic compounds was indicated by a color change to green or blue. The total phenolic content was determined using the Singleton and Rossi method with Folin–Ciocalteu reagent. A total of 0.5 g of extract was dissolved in a water–methanol solution (1:1) to obtain a final volume of 10 mL and the mixture was homogenized. Then, 0.2 mL of the sample solution was transferred into a test tube, followed by the addition of 15.8 mL distilled water and 1 mL of Folin–Ciocalteu reagent. The mixture was incubated under dark conditions for 8 minutes, after which 3 mL of 20%  $\text{Na}_2\text{CO}_3$  solution was added. The solution was mixed thoroughly and incubated again in the dark at room temperature for 2 hours. Absorbance was then measured at 765 nm using a Genesys

30-Vis spectrophotometer, and total phenolic content was calculated based on a previously prepared gallic acid standard curve.

### **Ferric Reducing Antioxidant Power (FRAP) Assay.<sup>16</sup>**

The assay was initiated by preparing the FRAP reagent, after which the absorbance of the control was measured. The absorbance of chrysanthemum flower extract was then measured at concentrations of 10, 15, 20, 25, and 30  $\mu\text{g}/\text{mL}$ . Trolox, at the same concentrations was used as a reference standard. The assay was performed in triplicate, and absorbance was measured at 594 nm using a Genesys 30-Vis spectrophotometer. The percentage of inhibition was calculated using the following formula:

Inhibition (%) =

$$\frac{\text{Abs.Control}(\lambda) - \text{Abs.Sample}(\lambda)}{\text{Abs.Control}(\lambda)} \times 100\%$$

### **Brine Shrimp Lethality Test (BSLT).<sup>17</sup>**

The test solution was prepared by dissolving 0.21 g of chrysanthemum flower extract in dimethyl sulfoxide (DMSO), followed by the addition of two drops of yeast solution and 10 mL of seawater in a beaker glass. The resulting solution was then transferred into test tubes to obtain concentrations of 5, 10, 15, 20, and 25  $\text{g}/\text{mL}$ . Each tube was adjusted to a final volume of 1 mL with seawater.

Subsequently, *Artemia salina* larvae (n=10) were added to each test tube, followed by filtered seawater to a total volume of 2 mL, yielding final concentrations of 5, 10, 20, 30, 40, and 50 g/mL. The assay was performed in duplicate.

The tubes containing the shrimp larvae were exposed to light for 24 hours. After incubation, the number of live and dead larvae in each test solution was recorded. Seawater alone and seawater containing DMSO served as negative controls.

The percentage of larval mortality was calculated using the following formula:

$$\text{Mortality (\%)} = \frac{\text{number of dead larvae}}{\text{total number of larvae}} \times 100\%$$

## RESULTS AND DISCUSSION

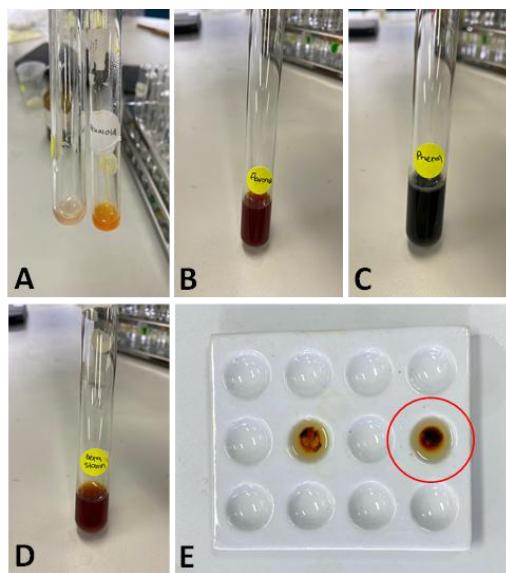
Various assays can be performed to screen phytochemical compounds in natural products, including tests for alkaloidss,

terpenoids, flavonoids, and phenolics, among others.<sup>18</sup> Phytochemical screening of the chrysanthemum flower extract revealed the presence of several compounds (Table 1), including alkaloids (Figure 1A), flavonoids (Figure 1B), phenols (Figure 1C), betacyanins (Figure 1D), and terpenoids (Figure 1E), .

These findings are consistent with previous studies reporting the presence of flavonoids, terpenoids, and alkaloids in the Chrysanthemum extracts obtained through maceration process.<sup>19</sup> Among the various secondary metabolites found in plants, flavonoids are the most abundant and widely distributed. These compounds provide numerous health benefits, including antimicrobial and anti-inflammatory properties. Furthermore, flavonoids are an important source of natural antioxidants, possessing a strong capacity to scavange free radicals.<sup>20,21</sup>

**Table 1** Phytochemical compounds detected in *Chrysantemum morifolium* flower extract

Phytochemical Compounds	Reagent/Method	Test Result
Alkaloids	Mayer	+
Flavonoids	NaOH	+
Terpenoids	Liebermann-Burchard	+
Anthocyanins-Betacyanins	NaOH	+
Phenolics	Folin-Ciocalteu	+



**Figure 1** Qualitative phytochemical screening of *Chrysanthemum morifolium* flower extract. (A) Alkaloids, (B) flavonoids, (C) phenols, (D) betacyanins, (E) terpenoids (indicated by red circle).

In addition to flavonoids, phenolic compounds constitute another widely distributed group of secondary metabolites. In this study, both qualitative and quantitative analyses were conducted to assess phenolic content. The qualitative test, conducted using the Folin–Ciocalteu method, showed a positive result (Figure 1C), which was then followed by quantitative determination of the total phenolic content in chrysanthemum flower extract, yielding a value of  $176.49 \pm 0.85$  mg GAE/g (Table 2). Gallic acid was used

as the standard compound due to its stability and frequent application as a reference phenolic compound.<sup>22</sup>

Phenolic compounds are well recognized for their health-promoting properties, particularly their protective effects against oxidative damage.<sup>23</sup> Based on these phytochemical screening results, the chrysanthemum flower extract demonstrates strong potential as a natural antioxidant source, attributable to its rich metabolite composition.

**Table 2** Total phenolic content of *Chrysanthemum morifolium* flower extract

Mean Absorbance	Total Phenolic Content (µg/mL)	Total Phenolic Content (mgGAE/gram)
$0.485 \pm 0.002$	$5330 \pm 26.45$	$176.49 \pm 0.85$

In this study, the antioxidant activity of chrysanthemum flower extract was evaluated using the Ferric Reducing Antioxidant Power (FRAP) method, which measures the ability of a sample to reduce  $\text{Fe}^{3+}$  ions to  $\text{Fe}^{2+}$ .<sup>24</sup> Based on the FRAP analysis, the  $\text{IC}_{50}$  value of chrysanthemum flower extract was calculated from the linear regression equation  $Y = 0.9788 X + 39.11$  with  $R^2 = 0.9888$  yielding 11.13  $\mu\text{g/mL}$  (Table 3). Trolox, a vitamin E analog commonly used as a reference antioxidant standard,<sup>25</sup> was also tested and yielded an  $\text{IC}_{50}$  of 14.17  $\mu\text{g/mL}$ , derived from the linear regression equation  $Y = 1.080 X + 34.70$  with  $R^2 = 0.9698$ .

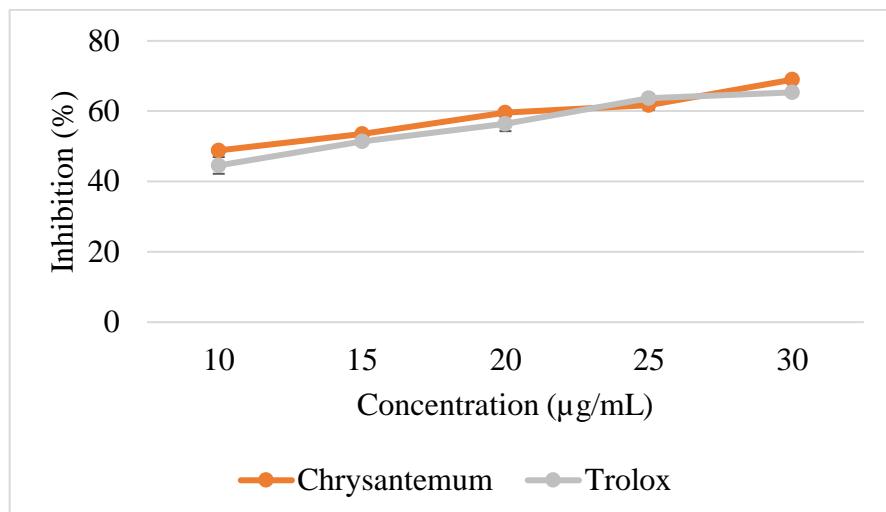
In antioxidant assays, the  $\text{IC}_{50}$  value indicates the concentration required to inhibit 50% of free radical activity, with lower values corresponding to stronger antioxidant activity.<sup>26</sup> Antioxidant strength is classified as follows:  $\text{IC}_{50} < 50$  ppm (very strong),  $\text{IC}_{50} 50\text{--}100$  ppm (strong),  $\text{IC}_{50} 101\text{--}150$  ppm (moderate),  $\text{IC}_{50} 150\text{--}200$

ppm (weak), and  $\text{IC}_{50} > 200$  ppm (very weak).<sup>27</sup> Accordingly, the chrysanthemum flower extract demonstrated very strong antioxidant capacity, which was comparable to that of the reference standard Trolox (Figure 2).

These findings are consistent with previous studies reporting strong antioxidant activity in chrysanthemum flowers.<sup>6,28</sup> Variations in antioxidant capacity across studies may arise from differences in experimental design, plant origin, or extract composition.<sup>29</sup> Chrysanthemum exerts its antioxidant effects by modulating the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), and by targeting arginine biosynthesis and purine metabolism pathways.<sup>30</sup> In addition, other studies suggest that its antioxidant properties in herbal cosmetics may be associated with tyrosinase inhibition.<sup>6</sup>

**Table 3** Antioxidant activity of *Chrysanthemum morifolium* flower extract measured by FRAP method

Concentration of Chrysanthemum Extract ( $\mu\text{g/mL}$ )	Absorbance (mean $\pm$ SD)	Inhibition (%)	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
10	0.176 $\pm$ 0.008	48.85 $\pm$ 0.77	
15	0.194 $\pm$ 0.014	53.59 $\pm$ 0.64	
20	0.223 $\pm$ 0.012	59.64 $\pm$ 0.48	11.13
25	0.239 $\pm$ 0.016	61.77 $\pm$ 1.38	
30	0.290 $\pm$ 0.020	68.96 $\pm$ 0.28	



**Figure 2** Inhibition comparison between *Chrysanthemum morifolium* flower extract and Trolox in the FRAP assay.

A toxicity assay was also performed in this study, using the Brine Shrimp Lethality Test (BSLT), a simple, inexpensive, and practical preliminary bioassay method used to evaluate the efficacy of phytochemical constituents.<sup>17</sup> A compound is generally considered potentially toxic when its LC<sub>50</sub> value is below 1000 μg/mL.<sup>31</sup> The chrysanthemum flower extract tested in this study showed an LC<sub>50</sub> of 15.49 μg/mL, calculated from the linear regression equation  $Y = 97.085X - 65.575$  ( $R^2 = 0.9919$ ) (Table 4). This result indicates that a minimum concentration of 15.49 μg/mL was sufficient to induce 50% mortality in *Artemia salina* larvae after 24 hours of incubation.

In contrast, the control groups- larvae placed either in seawater alone or in seawater containing DMSO-exhibited no mortality. These findings suggest that chrysanthemum flower extract from Sukabumi possesses significant toxicity potential, which may be associated with antimitotic activity. Consistent with this observation, previous studies have reported cytotoxic effects of chrysanthemum flower extract against several cancer cell lines, including A549 (lung), HepG2 (liver), and MCF-7 (breast) cancer cells.<sup>32</sup> Furthermore, flavonoids derived from chrysanthemum have been shown to inhibit tumor cell proliferation and induce apoptosis,<sup>21</sup> supporting its potential role in tumor growth regulation.

**Table 4** BSLT result of *Chrysanthemum morifolium* flower extract

Concentration ( $\mu\text{g/mL}$ )	Log Concentration ( $\mu\text{g/mL}$ )	Mortality (%)	$\text{LC}_{50}$ ( $\mu\text{g/mL}$ )
5	0.70	6.12	
10	1.00	25.64	
20	1.30	60.00	
30	1.48	79.59	15.49
40	1.60	91.67	
50	1.70	98.67	

## CONCLUSION

The present study demonstrates that chrysanthemum flower extract from Sukabumi, West Java, contains a diverse range of phytochemical constituents, exhibits very strong antioxidant activity, and shows pronounced cytotoxic potential indicative of both antimitotic and antitumor properties. These findings suggest that the Sukabumi variety of *Chrysanthemum* flowers may serve as a promising natural source of antioxidants and represents a potential candidate for further investigation in anticancer research.

## CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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