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ORIGINAL ARTICLE

Investigating the Anti-Inflammatory Potential of Ethanol Extracts from Purple Chrysanthemum Flowers

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Abstract

Indonesia has high biodiversity, with around 9,600 species of flora possessing medicinal properties. One such plant is the purple chrysanthemum (*Chrysanthemum morifolium*), which is rich in bioactive compounds with anti-inflammatory potential. The purple chrysanthemum is used in traditional medicine to treat various diseases and has demonstrated significant anti-inflammatory activity *in vitro*. Given the high prevalence of inflammatory diseases in Indonesia and the side effects associated with conventional drugs, this plant is considered a promising alternative therapy that may be safer and more effective. The aim of this study was to identify the phytochemical compounds and evaluate the anti-inflammatory activity of an ethanol extract from purple chrysanthemum flowers (*Chrysanthemum morifolium*) *in vitro*. The extract was prepared using 96% ethanol, and its anti-inflammatory activity was assessed using the red blood cell membrane stabilization method. Blood samples were collected from Wistar rats. The inhibitory concentration (IC_{50}) values of the extract and Diclofenac Sodium were 203.08 ppm and 224.04 ppm, respectively. These findings indicate that the ethanol extract of purple chrysanthemums exhibits anti-inflammatory activity

Introduction

Chrysanthemum morifolium, commonly known as the chrysanthemum flower, is a plant with significant potential as a medicinal herb in Indonesia. Chrysanthemum may assist in the treatment of various diseases, including pneumonia, cancer, and hypertension, and it exhibits analgesic properties, lowers blood pressure, and demonstrates anti-inflammatory activity [1–3]. This is attributed to the presence of bioactive phytoconstituents, such as flavonoids, glycosides, polyphenols, essential oils, terpenoids, steroids, and polysaccharides [4].

The incidence of diseases involving inflammatory processes in Indonesia remains relatively high. According to the 2018 Basic Health Research (Riset Kesehatan Dasar) conducted by the Indonesian Ministry of Health, the prevalence of inflammatory diseases includes asthma (2.4%), cancer (1.79%), diabetes mellitus (DM) (1.5%), and joint diseases (7.3%). In North Sulawesi Province, the prevalence of gout reached 8.35%, which is higher than the national average. Additionally, the Indonesian Ministry of Health reported that asthma affected approximately 4.5% of the population, equivalent to around 11,179,032 individuals [5]. Data from the Global Cancer Observatory (Globocan) indicated that in 2022, Indonesia recorded over 408,661 new cancer cases, with 242,099 related deaths. The population-based incidence of Inflammatory Bowel Disease (IBD) in Indonesia was reported at 0.76 per 100,000 individuals [6].

Data from the Social Security Administering Body for Health (Badan Penyelenggara Jaminan Sosial/BPJS Kesehatan) indicates that the expenditure for covering participant claims reached IDR 1.5 trillion in 2014, increased to IDR 2.2 trillion in 2015, and amounted to approximately

IDR 2.3 trillion in 2016 [7]. The International Diabetes Federation (IDF) estimates that the annual global expenditure for diabetes treatment is around USD 850 billion. This highlights the significant socio-economic impact of diabetes, posing a threat to national productivity and economic stability, particularly in low- and middle-income countries [7].

Numerous medications regulate the inflammatory response, including steroids and non-steroidal anti-inflammatory drugs (NSAIDs); however, both classes of drugs have significant side effects. Steroids can lead to musculoskeletal issues and cardiovascular complications, while NSAIDs may affect the stomach, kidneys, and increase the risk of gastric ulcers [8]. Purple chrysanthemum (Chrysanthemum morifolium) is known to contain bioactive compounds with potential antioxidant, antimicrobial, and anti-inflammatory properties. A study by Liu et al., which identified 114 compounds from six samples of essential oils extracted from Chrysanthemum morifolium, revealed that the primary constituents include flavonoids, which may play a direct role in anti-inflammatory activity [9]. Furthermore, research by Niharika et al., demonstrated that the essential oil content of chrysanthemum flowers exhibits anti-inflammatory effects, as evaluated through in vitro cell experiments. The findings indicated that it effectively reduced inflammation in THP-1 cells induced by heat-killed *Propionibacterium acne*s suspension [2].

A study conducted by Liu et al. [10] evaluating the effects of ethanol extract of *Chrysanthemum morifolium* Ramat. (CEE) on lipopolysaccharide-induced acute lung injury in rats demonstrated that CEE could ameliorate pulmonary histopathological damage, reduce the lung wet-to-dry weight ratio and lung index, inhibit the increase in white blood cell, lymphocyte, and neutrophil counts, and suppress elevated levels of TNF-α and IL-6. Additionally, research by Ono et al. [11], which examined *Chrysanthemum morifolium* extract (CME) using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay, revealed that CME treatment alleviated doxorubicin (DOX)-induced cardiotoxicity by suppressing apoptosis.

Several previous studies have demonstrated that chrysanthemum (*Chrysanthemum morifolium*) contains flavonoid and phenolic compounds with anti-inflammatory activity through the modulation of the NF-κB pathway and inhibition of the COX-2 enzyme [12]. However, most of these studies have been limited to *in vitro* assays using cellular models. Furthermore, there is a lack of research specifically exploring the ethanol extract of purple chrysanthemum in the context of inflammation inhibition caused by pathogenic bacterial infections. The research method employed is in vitro, which is essential for evaluating the anti-inflammatory potential of a substance prior to clinical trials. This method aids in understanding the mechanisms of anti-inflammatory action at the cellular and molecular levels, and allows for direct measurement of effects without the risks of side effects associated with in vivo models [13].

This study aims to investigate the anti-inflammatory activity of the ethanol extract of purple chrysanthemum (*Chrysanthemum morifolium*), with the expectation of making a significant contribution to the development of safer and more effective alternative treatments for inflammatory conditions.

Materials and Methods

Plant Determination

The purple chrysanthemum (Chrysanthemum morifolium) flowers utilized in this study were initially collected and identified by a taxonomist at CV BIOVINA: Laboratory. This identification process involved verifying the plant's scientific classification based on its morphological traits.

Sample Preparation

The purple chrysanthemum samples were collected from Tomohon City, North Sulawesi, at an altitude of 780 meters. The flowers were washed, dried for three days, and then oven-dried at 60°C for 24 hours. Once dried, the flowers were ground using a blender and sieved through a 40 mesh to produce a dry, fine, and uniform simplicia powder.

The blood used in this study was obtained from Wistar rats (Rattus norvegicus), which served as the test animals. Prior to sampling, the rats underwent a 7-day adaptation period in the laboratory to ensure their physiological stability. Blood collection was performed using the Cardiac Puncture method, with the rats being anesthetized with a general anesthetic, such as ketamine-xylazine, to prevent pain and movement during the procedure. This technique was employed to obtain a sufficient volume of blood in a single collection, following laboratory animal welfare standards [14]. The study received ethical approval from the Research Ethics Committee of the Faculty of Agriculture, Sam Ratulangi University, under the ethics code 08/KEH-UNSRAT/REC/2024, in accordance with ethical guidelines for the use of laboratory animals in scientific research.

Extraction Process

The extraction of purple chrysanthemum flowers was conducted using an ultrasonic method with 96% ethanol as the solvent. A total of 80 grams of flower powder was mixed with 800 mL of ethanol and extracted at a frequency of 42 kHz and a temperature of 50°C for 30 minutes. After filtration with Whatman paper, the mixture was evaporated using a rotary evaporator at a pressure of 24 kPa and a temperature of 50°C.

Ultrasound with frequencies exceeding 20 kHz has demonstrated the ability to efficiently extract bioactive components from natural products. Based on various studies, it is generally recommended to use low frequencies (20–40 kHz) for extracting flexible materials such as plant-based substances and algae [15]. Zhao et al. reported that the optimal extraction conditions include 70% ethanol, a solid-to-liquid ratio of 1:30, and a temperature of 50°C [16]. Findings by Stevanato et al. indicated that the optimal extraction time in ultrasonic-assisted extraction (UAE) ranges between 5–30 minutes, during which the extraction rate of oil increases over time [17]. The obtained extract was filtered through Whatman No.1 filter paper and concentrated using a rotary evaporator until the solvent was completely removed. The resulting concentrated extract was then stored in a dark glass bottle at -20°C until further analysis. This storage temperature was selected based on a study by Markhali et al., which showed that stability tests indicated -20°C as the optimal condition for extract stability, while storage at 25°C was the least favorable, particularly for oleuropein and total phenolic content (TPC). The extract was stored for no more than seven days before analysis to ensure the stability of its active compounds [18].

Phytochemical Screening

Phytochemical screening of the ethanol extract of purple chrysanthemum (*Chrysanthemum morifolium*) included several tests: alkaloids were tested with chloroform and ammonia, resulting in a precipitate; flavonoids were tested with heating and HCl, showing a red-orange color; saponins were tested by shaking in hot water, producing stable foam; tannins were tested with $FeCl_3$, indicating a green or blue color; triterpenoids and steroids were tested with CH_3COOH and H_2SO_4, yielding red and green colors; and phenols were tested with $FeCl_3$, showing a greenish-black color.

Preparation of Required Solutions and Red Blood Cell Suspension

A phosphate buffer at pH 7.4 (0.15 M) was prepared by dissolving 13.35 grams of disodium hydrogen phosphate and 4.14 grams of sodium dihydrogen phosphate in distilled water, bringing the total volume to 500 mL (0.15 M) in a volumetric flask. The solution was sterilized by autoclaving at 121°C for 2 hours. An isotonic solution was prepared by dissolving 0.85 grams of NaCl in the phosphate buffer at pH 7.4 (0.15 M) to reach a final volume of 250 mL. A

hypotonic solution was prepared by dissolving 0.36 grams of NaCl in the phosphate buffer at pH 7.4 (0.15 M) to reach a final volume of 100 mL. Fresh blood collected in an EDTA tube (10 mL) was centrifuged, and the resulting supernatant was separated using a sterile micropipette. The remaining pellet was washed with isotonic solution and then centrifuged again. This process was repeated three times until the isotonic solution became clear [19].

Preparation of Extract Concentrations, Sodium Diclofenac, and Determination of Maximum Wavelength

The extract concentration was prepared by dissolving 100 mg of ethanol extract from purple chrysanthemum flowers in isotonic solution, bringing the total volume to 100 mL. For sodium diclofenac, 100 mg of sodium diclofenac powder was dissolved in 100 mL of isotonic solution. Both solutions were then diluted into four serial concentrations: 50 ppm, 100 ppm, 200 ppm, and 400 ppm. The selection of these concentrations in this study was based on previous studies that evaluated the antibacterial effects of plant extracts containing flavonoids and phenolics. Research by Tavita et al. showed that at a concentration of 50 ppm, red blood cell membrane stabilization reached only 28.77%, indicating that this concentration did not have a significant effect. A study by Anggara et al. showed that at a concentration of 400 ppm, the methanol fraction of tamarind leaf exhibited a red blood cell membrane stability of 88.9%, which was comparable to the positive control (89.9%).

Preparation of Test Solution, Positive Control, and Negative Control

The test solution consisted of 1 mL of phosphate buffer pH 7.4 (0.15 M), 0.5 mL of red blood cell suspension, 0.5 mL of sample solution with various concentrations, and 2 mL of hypotonic solution. The positive control solution consisted of a mixture of 1 mL of phosphate buffer pH 7.4 (0.15 M), 0.5 mL of red blood cell suspension, 1 mL of sodium diclofenac solution with various concentrations, and 2 mL of hypotonic solution. The negative control solution consisted of 1 mL of phosphate buffer pH 7.4 (0.15 M), 0.5 mL of red blood cell suspension, 2 mL of hypotonic solution, and 1 mL of isotonic solution.

Anti-Inflammatory Activity Testing

The anti-inflammatory activity was assessed using the Red Blood Cell Membrane Stabilization method, beginning with the preparation of a phosphate buffer at pH 7.4 (0.15 M) and isotonic and hypotonic solutions, all of which were sterilized by autoclaving. A red blood cell suspension was prepared from fresh blood that was centrifuged and washed with isotonic saline until clear. Concentrations of the ethanol extract of purple chrysanthemum and sodium diclofenac were prepared and then diluted to various concentrations for further testing.

The maximum wavelength measurement was conducted using a UV-Vis spectrophotometer after incubating a solution consisting of phosphate buffer, red blood cell suspension, sodium diclofenac solution, and hypotonic saline. The test solution, positive control, and negative control were incubated and centrifuged before measuring their absorbance. This process is crucial for determining the effectiveness of the extract in stabilizing red blood cell membranes.

The IC_{50} value was calculated by plotting concentration against % stability of the solution, indicating how effectively the extract inhibits inflammatory activity. The stability of the test solution and sodium diclofenac provides insight into the anti-inflammatory potential of the tested extract, calculated using the formula comparing the absorbance of the test solution with the negative control, as shown in Equation 1.

$$\%\ Stability = 100 - \frac{Abs\ of\ test\ solution}{Abs\ of\ negative\ control\ solution}\ x\ 100\% \quad (1)$$

Data Analysis

The percentage stability data of the test solution and sodium diclofenac were analyzed using SPSS, where the Shapiro-Wilk test was employed to assess normality and the Levene test was used to evaluate homogeneity. If the data were normally distributed and homogeneous, one-

way analysis of variance (ANOVA) was performed at a 95% confidence level, and if significant differences were found, it was followed by the least significant difference (LSD) test using the Tukey method. However, if the data were not normally distributed and homogeneous, the analysis proceeded with the Kruskal-Wallis non-parametric test, and if significant differences were identified, the Dunn-Bonferroni post hoc test was conducted.

Results and Discussion

In this study, the samples used were purple chrysanthemum flowers (*Chrysanthemum morifolium*) collected from Kakaskasen Tiga Village, North Tomohon District, Tomohon City, North Sulawesi Province, at an altitude of 780 meters above sea level. To preserve the compound content in the flowers, the samples consisted of 693 grams of fresh flowers. Following a series of processes to prepare simplicia, including washing, slicing, drying, and grinding, a total of 81 grams of purple chrysanthemum powder was obtained [20].

The extraction method employed in this study is ultrasonic extraction for purple chrysanthemum flowers (*Chrysanthemum morifolium*). This method, known as ultrasonic-assisted extraction (UAE), is non-destructive and efficient, allowing for a faster extraction process of organic compounds using 96% ethanol as the solvent [21]. Ethanol was selected as the solvent due to its lower toxicity compared to other solvents, affordability, and safety for pharmaceutical and food products [22]. This process resulted in 12 grams of concentrated extract. The yield of the extract was calculated using a formula indicating that the percentage yield of the extract from the initial weight was 14.8%.

Phytochemical screening was performed to identify the active compounds present in purple chrysanthemum flowers (Chrysanthemum morifolium). Table 1 presents the results of the tests, which show the presence of several bioactive compounds, including flavonoids, triterpenoids/steroids, tannins, phenolics, and alkaloids.

Table 1. Results of phytochemical testing of the ethanol extract of purple chrysanthemum flowers.

No.	Examination	Reagent	Result	Description
1	Phenolics	$FeCl_3$	+	Green color formed
2	Flavonoids	HCl	+	Brick red color formed
		Magnesium Powder		
3	Saponins	Aquades	-	No stable foam present
4	Tannins	$FeCl_3$	+	Dark green appearance
5	Steroids/Triterpenoids	H_2SO_4	+	Bluish-green appearance
		100% Glacial Acetic Acid		
6	Alkaloids	Wagner	+	Red precipitate formed
		Mayer	+	White precipitate formed
		Dragendorf	+	Orange precipitate formed

Description: (+): indicates the presence of a compound group, (-) : indicates the absence of a compound

Phytochemical testing on purple chrysanthemum flowers (*Chrysanthemum morifolium*) yielded positive results for several significant active compounds. Alkaloids were detected by the presence of a white precipitate in the Mayer test, an orange-brown precipitate in the Dragendorff test, and a brown precipitate in the Wagner test [23]. Furthermore, flavonoids also exhibited positive results, indicated by the appearance of an orange-red color in the solution [24]. Triterpenoids and steroids were identified by a red and green color, respectively, during testing. Tannins produced positive results, evidenced by the appearance of a green to blue or dark green color, while phenolics demonstrated positive results with a dark green color. However, the saponin test yielded negative results, characterized by the absence of stable foam formation for a minimum of 10 minutes [25].

In this study, the anti-inflammatory activity was evaluated using the in vitro red blood cell membrane stabilization method, as the structure of red blood cell membranes is similar to that

of lysosomal membranes involved in the inflammatory process. This method is also practical due to the ease of obtaining and isolating red blood cells. Compared to other methods such as COX-2 enzyme inhibition or nitrite production inhibition, which often require the use of chemical reagents or more expensive equipment, the red blood cell membrane stabilization method is simpler, more accessible, and requires minimal raw materials. However, this method often uses hypotonic induction to test membrane stability, which may not fully reflect the inflammatory conditions occurring in the body [26]. The use of hypotonic solutions can cause non-specific cell lysis, so the resulting data may not entirely represent the mechanism of action of anti-inflammatory compounds. Measurements were conducted using a UV-Vis spectrophotometer at a wavelength of 576 nm, with sodium diclofenac serving as a positive control known for its effectiveness and lower side effects compared to other anti-inflammatory drugs [27,28].

Table 2 presents the absorbance measurements of purple chrysanthemum flower extract and Sodium Diclofenac. After the measurements were taken, the absorbance data were calculated to determine the percentage of red blood cell membrane stability using the stabilization percentage formula. The anti-inflammatory activity was assessed using the inhibition concentration parameter (IC_{50}).

Table 2. Results of absorbance measurement of ethanol extract of purple chrysanthemum flowers and sodium diclofenac using the red blood cell membrane stabilization method.

Sample	Concentration (ppm)	Mean ± SD	%Stability
Ethanol Extract of Purple Chrysanthemum Flowers	50	0.66 ± 0.002	25.30
	100	0.48 ± 0.001	45.83
	200	0.46 ± 0.001	48.35
	400	0.24 ± 0.000	73.01
Sodium Diclofenac	50	0.72 ± 0.001	18.81
	100	0.67 ± 0.000	24.81
	200	0.39 ± 0.003	56.23
	400	0.22 ± 0.000	75.71

The percentage stabilization data were plotted on a graph with the test solution concentration on the x-axis (abscissa) and the percentage stabilization on the y-axis (ordinate). Figure 1 shows the red blood cell membrane stabilization curve for purple chrysanthemum flower extract, with the equation obtained being y = 0.1201x + 25.61 and a correlation coefficient (R^2) of 0.9045. Meanwhile, Figure 2 represents the red blood cell membrane stabilization curve for sodium diclofenac, with the equation obtained being y = 0.1674x + 12.495 and a correlation coefficient (R^2) of 0.9337. The IC_{50} value for ethanol extract of purple chrysanthemum flowers was 203.08 ppm, while the IC_{50} value for sodium diclofenac was 224.04 ppm. Based on the results, the IC_{50} values for both sodium diclofenac and ethanol extract of purple chrysanthemum flowers fall into the moderate category. Anti-inflammatory activity is classified as very active if the IC_{50} value is less than 50 ppm, active if it is in the range of 50 to 100 ppm, moderate if between 101 to 250 ppm, weak if between 250 to 500 ppm, and inactive if the IC_{50} value exceeds 500 ppm [29].

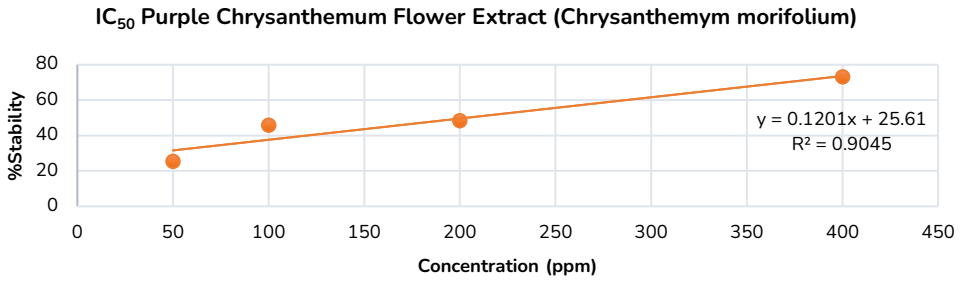

Figure 1. Stabilization curve of erythrocyte membranes from purple chrysanthemum flower extract at various concentrations against hypotonic solution induction.

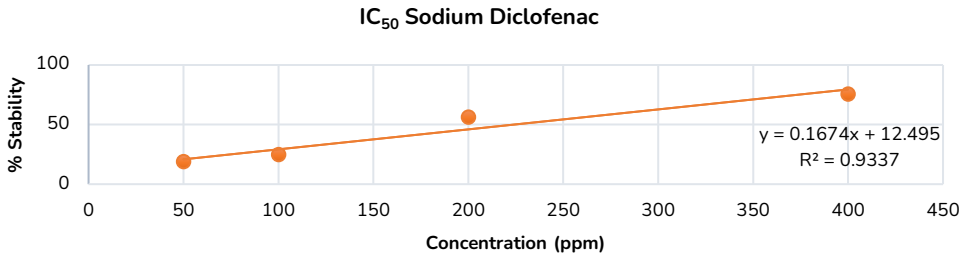

Figure 2. Stabilization curve of erythrocyte membranes from sodium diclofenac at various concentrations against hypotonic solution induction.

The results of this study indicate that the ethanol extract of purple chrysanthemum flowers exhibits anti-inflammatory activity, with an IC_{50} value of 203.08 ppm, which is relatively close to the IC_{50} of Sodium Diclofenac (224.04 ppm). Previous studies have shown that several other plant extracts also possess comparable anti-inflammatory potential. Findings by Fadli et al. reported that the extract of *Curcuma aeruginosa rhizome* exhibited an IC_{50} of 171 ppm in a cellular inflammation model, while grated turmeric extract was reported to have an IC_{50} of 158.3 ppm (Maharani et al., 2012). Additionally, studies on other chrysanthemum varieties have also demonstrated varying anti-inflammatory potential [30]. A study by Shao et al. revealed that the extract of chrysanthemum flowers (Chrysanthemi indic Flos) exhibited significant anti-inflammatory activity. In this study, the extract demonstrated an IC_{50} value for cyclooxygenase-2 (COX-2) inhibition of 1.06 ± 0.01 µg/mL [31]. These findings suggest that the bioactive compound content, such as flavonoids and phenolics, may vary among chrysanthemum varieties, thereby influencing their pharmacological activity.

Table 3. Normality test using the Shapiro-Wilk test.

Treatment		*Shapiro-Wilk*		
	Concentration	Statistic	df	Sig.
Stability Percentage	50 ppm BU	0.923	3	0.464
	100 ppm BU	1.000	3	0.995
	200 ppm BU	0.750	3	<0.001
	400 ppm BU	0.796	3	0.106
	50 ppm ND	1.000	3	0.995
	100 ppm ND	0.750	3	<0.001
	200 ppm ND	0.993	3	0.842
	400 ppm ND	0.750	3	<0.001

Description: BU: Purple chrysanthemum flower extract; ND: Sodium diclofenac / positive control.

Table 4. Kruskal-Wallis data analysis results.

Solution	Concentration (ppm)	*p-value*	Interpretation
Ethanol Extract of Purple Chrysanthemum	50	0.002	There is a difference between groups
	100		
	200		
	400		
Sodium Diclofenac	50		
	100		
	200		
	400		

The 400 ppm concentration, which represents the highest concentration of the sample, exhibited a maximum stabilization capacity of 73.01%. This finding is further supported by statistical analysis. As presented in Table 3, normality testing using the Shapiro-Wilk method indicated that the treatment groups were not normally distributed ($p \leq 0.05$), necessitating further analysis with the Kruskal-Wallis test. Table 4 presents the results of the Kruskal-Wallis test, which showed significant differences ($p \leq 0.05$). Consequently, a post hoc analysis was

conducted using the Dunn-Bonferroni test to identify significant differences between the concentrations of the extract and sodium diclofenac as the positive control.

As shown in Table 5, higher concentrations of the ethanol extract of purple chrysanthemum flowers exhibited a greater effect on stabilizing red blood cell membranes. A significant difference ($P<0.05$) was observed between the 50 ppm positive control solution and the test solutions at concentrations of 200 ppm and 400 ppm. Additionally, a significant difference ($P<0.05$) was found between the 50 ppm and 100 ppm test solutions. Based on the data analysis, the test solution at a concentration of 400 ppm demonstrated the highest membrane stabilization potential, suggesting that higher extract concentrations produce effects comparable to sodium diclofenac, which served as the positive control. These findings indicate that the extract at 400 ppm exhibits significantly stronger anti-inflammatory activity compared to lower concentrations. This outcome provides a basis for further research on the optimal dosage for therapeutic formulations.

Table 5. Post Hoc test results.

	BU1	BU2	BU3	BU4	ND1	ND2	ND3	ND4
BU1		0.603	0.298	0.038	0.298	0.603	0.119	0.009
BU2	0.603		0.603	0.119	0.119	0.298	0.298	0.038
BU3	0.298	0.603		0.298	0.038	0.119	0.603	0.119
BU4	0.038	0.119	0.298		0.002	0.009	0.603	0.603
ND1	0.298	0.119	0.038	0.002		0.603	0.009	<0.001
ND2	0.603	0.298	0.119	0.009	0.603		0.038	0.002
ND3	0.119	0.298	0.603	0.603	0.009	0.038		0.298
ND4	0.009	0.038	0.119	0.603	<0.001	0.002	0.298	

The test results indicate that the IC_{50} value of the ethanol extract of purple chrysanthemum flowers is 203.08 ppm, while the IC_{50} of sodium diclofenac is 224.044 ppm. This IC_{50} value suggests that the extract exhibits anti-inflammatory potential comparable to sodium diclofenac, as the concentration required to achieve 50% inflammation inhibition is not significantly different. A study by Armaini et al. on the determination of astaxanthin levels, anti-inflammatory testing, and cytotoxicity against MCF-7 breast cancer cells found that aspirin had an IC_{50} value of 120.561 mg/L [32]. Another study by Mitchell et al., which investigated the bioactive compound andrographolide, reported that paracetamol, used as a positive control, had an IC_{50} value of 491.24 ppm. These findings suggest that the purple chrysanthemum flower extract possesses anti-inflammatory potential that is competitive with sodium diclofenac and approaches the effectiveness of aspirin.

The anti-inflammatory effects of purple chrysanthemum flower extract are suspected to arise from secondary metabolites such as flavonoids, phenolics, and tannins. Flavonoids exert their anti-inflammatory mechanism by inhibiting the cyclooxygenase (COX) and lipoxygenase enzymes, thereby reducing the biosynthesis of prostaglandins and leukotrienes. This activity also inhibits mucus secretion that protects the gastric lining and reduces the body's inflammatory response by preventing leukocyte accumulation. Additionally, flavonoids suppress histamine release from mast cells and neutrophil degranulation, which contributes to the reduced release of arachidonate by neutrophils [33]. Steroids also exhibit anti-inflammatory potential by inhibiting phospholipase enzymes in the arachidonic acid pathway. Tannins act as antioxidants and anti-inflammatory agents, as their hydroxyl (-OH) groups can donate hydrogen atoms to free radicals, converting them into non-radical compounds [34]. Meanwhile, phenolic compounds can inhibit oxidation and reduce superoxide, peroxyl, and hydroxyl radicals. Moreover, phenols can inhibit the activity of cyclooxygenase receptors, which are key enzymes involved in the inflammatory response.

Conclusions

This study revealed that the ethanol extract of purple chrysanthemum flowers (Chrysanthemum morifolium) contains bioactive compounds such as flavonoids, tannins, phenolics, triterpenoids/steroids, and alkaloids. The anti-inflammatory activity of the ethanol extract, measured through red blood cell membrane stabilization, demonstrated an IC_{50} value of 203.08 ppm, while the IC_{50} value for sodium diclofenac was 224.04 ppm. Based on these results, both the ethanol extract and sodium diclofenac fall within the moderate category of anti-inflammatory activity. Statistical analysis indicated that the difference in anti-inflammatory efficacy between the extract and the control was significant ($p < 0.05$), confirming that the extract exhibits anti-inflammatory activity. However, this study only assessed anti-inflammatory activity in vitro, necessitating further in vivo studies and methodologies that directly examine the mechanisms of inflammation, such as the inhibition of specific inflammatory mediators. Additionally, future research should explore the bioavailability, stability, and potential side effects of the extract in more complex biological systems.

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