Indonesian Impression Journal (JII)
Vol. 4, No. 2, February 2025
p-ISSN: 2828-1284 e-ISSN: 2810-062x
Website: https: //rivierapublishing.id/JII/ index.php / jii /index

IN VITRO EVALUATION OF THE ANTI-INFLAMMATORY POTENTIAL OF ETHANOL EXTRACT FROM PINK CHRYSANTHEMUM (CHRYSANTHEMUM MORIFOLIUM)

Gloria Prisca Susana Angelique Baya[1], Aaltje Ellen Manampiring[2], Fatimawali[3], Fona Dwiana Hermina Budiarso[4], Billy Johnson Kepel[5]
Sam Ratulangi University, Indonesia
gloriabaya011@student.unsrat.ac.id[1], aldakussoy@yahoo.com[2], fatimawali@unsrat.ac.id[3], fonabudiarso@unsrat.ac.id[4], billy.kepel@unsrat.ac.id[5]

Keywords
anti-inflammatory, pink chrysanthemum, ethanol extract, in vitro

Abstract
Inflammation is the body's normal response to injury. However, acute and chronic inflammatory reactions can lead to various diseases. Nonsteroidal anti-inflammatory drugs (NSAIDs) have been proven effective as anti-inflammatory agents, but excessive and long-term use of NSAIDs can cause various side effects. Pink chrysanthemum flowers (Chrysanthemum morifolium) are one of many medicinal plants used as traditional medicine due to their content of compounds such as flavonoids, triterpenoids, and steroids, which have anti-inflammatory properties and the potential to serve as alternatives to NSAIDs. To identify active compounds and analyze the anti-inflammatory activity of ethanol extract from pink chrysanthemum flowers (Chrysanthemum morifolium). This study is an experimental laboratory research. The extraction method used was Microwave-Assisted Extraction (MAE). The anti-inflammatory activity of the ethanol extract of pink chrysanthemum flowers was measured using the red blood cell membrane stabilization method. The tests were performed using a UV-Vis spectrophotometer at extract concentrations of 50 ppm, 100 ppm, 200 ppm, 400 ppm, and 800 ppm. Pink chrysanthemum flowers contain several phytochemical compounds, including alkaloids, flavonoids, tannins, triterpenoids/steroids, and phenolics. The anti-inflammatory activity measurement showed that higher extract concentrations resulted in lower absorbance values and increased stabilization percentages, indicating anti-inflammatory activity that protects red blood cell membranes from damage. The ethanol extract of pink chrysanthemum flowers contains several phytochemical compounds with potential anti-inflammatory activity. The extract at a concentration of 800 ppm demonstrated the highest membrane stabilization ability, with a stabilization rate of 59.459%.

Corresponding Author: Gloria Prisca Susana Angelique Baya
E-mail: gloriabaya011@student.unsrat.ac.id

INTRODUCTION

When an injury occurs, the body releases several substances in response. These substances include histamine, prostaglandins, cytokines, bradykinin, chemokines, leukotrienes and serotonin (Suryandari et al., 2021). The release of substances such as histamine results in dilation of blood vessels and increased permeability of the capillary walls so that phagocyte cells (leukocytes) can move towards the area to destroy substances that are considered harmful (phagocytosis) (Luthfianto et al., 2023). Excessive phagocytosis process can worsen inflammation. It is characterized by redness, swelling, heat, pain, and loss of tissue function (Ross et al., 2021). Inflammation is divided into two types, namely acute inflammation which is usually followed by immediate recovery, and there is also inflammation that develops into chronic when the causative agent is settled (Akrom, 2021).

Basically, inflammatory reactions are normal. But in fact, acute and chronic inflammatory reactions can cause various diseases. One example is rheumatoid arthritis (Arida et al., 2018). This disease has a reported prevalence rate of 20 percent of the world's population according to the World Health Organization (WHO) and also reaches 7.30% in Indonesia (Prince et al., 2015). In addition, there is another example, namely gout arthritis which is an inflammatory arthritis. In fact, in North Sulawesi itself, especially North Minahasa, has a prevalence rate of 29.2% (Hendayana, 2023). This prevalence also increases with age and unhealthy lifestyle which is directly proportional to the thick customs of Minahasa such as drinking alcoholic beverages and foods high in purines (red meat and certain seafood). Due to the high prevalence of diseases caused by inflammatory reactions, the use of anti-inflammatory drugs has also increased.

Administration of OAINS (nonsteroidal anti-inflammatory drugs) has been shown to be effective as an anti-inflammatory. However, excessive and long-term use of OAINS can cause various side effects such as cardiovascular tract disorders and kidney disorders (Arfania et al., 2023). This is one of the factors to consider when switching to traditional medicine. This is also related to Indonesia's nature, which is very rich in various plants that are useful as medicinal plants. Of the 30,000 types of plants in Indonesia, there are approximately 7,000 types of plants that are considered efficacious as medicinal ingredients. In Indonesia, this herbal treatment method has existed for generations in accordance with the prevailing customs in the community (Adiyasa & Meiyanti, 2021).

Pink chrysanthemum (Chrysanthemum morifolium) is one of the many medicinal plants that are used as herbal medicine by the Indonesian people (Prabawati et al., 2021). This flower contains several compounds such as flavonoids, triterpenoids and steroids that are efficacious as anti-inflammatory for the body. Wrong

One chemical compound in pink chrysanthemums that is beneficial to the body is flavonoids that have anti-inflammatory, antioxidant, antiallergic, and antiviral properties (Yuan et al., 2020). In addition, there are also triterpenoids that act as anti-inflammatory. This triterpenoid functions to prevent the enzyme cyclooxygenase, which converts arachidonic acid into prostaglandins (inflammatory mediators) (Wahdaniah et al., 2023).

Although many studies have been conducted on various anti-inflammatory properties of medicinal plants, special studies on the effectiveness of pink chrysanthemum flower extract

(Chrysanthemum morifolium) by testing anti-inflammatory activity using red blood cell stabilization method and extraction method by Microwave Assisted Extraction (MAE) method are still very limited. This research is expected to fill this gap and make new contributions in the field of herbal pharmacology, especially related to the potential of pink chrysanthemum flowers (Chrysanthemum morifolium) as a potential source of anti-inflammatory compounds.

RESEARCH METHOD

This study uses laboratory experimental methods, which aims to test the anti-inflammatory activity of ethanol extract of pink chrysanthemum flowers (Chrysanthemum morifolium) in vitro. Study uses a quantitative approach to analyze anti-inflammatory activity based on data from spectrophotometric testing.

Population and Sample

1. Population: Pink chrysanthemum plant (Chrysanthemum morifolium).
2. Sample: Pink chrysanthemums obtained from Kakaskasen Tiga, Tomohon City, North Sulawesi Province, with a geographical location of 770 meters above sea level.

Data Collection Techniques

Data is obtained through a series of laboratory tests which include:

1. Phytochemical screening for identification of active compounds (flavonoids, tannins, alkaloids, etc.).
2. Anti-inflammatory activity testing using erythrocyte membrane stabilization method with UV-Vis spectrophotometry.

Data Analysis Techniques

1. The data from the anti-inflammatory activity test results were analyzed using SPSS statistical software.
2. The normality test was carried out using Shapiro-Wilk, and the homogeneity test was carried out using the Levene test.
3. One-way variance analysis (ANOVA) is used to identify significant differences between groups.
4. If a significant difference is found, continue with the smallest real difference test (Tukey).

RESULTS AND DISCUSSION

Pink Chrysanthemum Flower Extraction Results

Pink chrysanthemum leaf simplicia powder of 98 grams was extracted by the Microwave Assisted Extraction method which uses 96% ethanol solvent with a ratio of 1:10 between simplicia powder and ethanol solvent. A total of 50 grams of simplicia powder is added with 500 ml of 96% ethanol solvent and then extracted using a microwave with a power of 700 watts for 1-6 minutes. The extraction solution is irradiated at regular intervals with a 1-minute radiation cycle and 2-minute turned off to keep the temperature no more than 80◦C. After irradiation, the solution is left at room temperature and filtered using filter paper until it produces a pink chrysanthemum filtrate and concentrated using a rotary vacuum evaporator. Once the filtrate has thickened, it is placed in an oven at 46◦C. From the evaporation process, 14,805 gr of thick extract was obtained.

$$\%\text{Rendement} = \frac{berat\ ekstrak\ yang\ didapat}{berat\ simplisia\ yang\ diekstraksi}\ x\ 100\%$$
$$= \frac{14{,}805}{50}\ x\ 100\% = 29{,}61\%$$

Based on the calculation above, the percentage value of the pink chrysanthemum extract was obtained of 29.61% which shows a good yield value. Because the higher the yield value, the higher the compound of interest and the more extracts produced.

Phytochemical Screening Results

The results of phytochemical screening of pink chrysanthemums from ethanol extract showed the presence of active compounds such as phenols, flavonoids, tannins, steroids/triterpenoids, and alkaloids so that they have great potential to have anti-inflammatory activity. The results of the phytochemical screening examination can be seen in the following Table 1:

Table 1. Phytochemical Screening Results

No.	Examination	Reagents	Result	Information
1.	Alkaloids	Wagner	+	No brown deposits formed
		Mayer	-	White deposits form
		Dragendorff	+	Orange deposits are formed
2.	Flavonoids	Hcl and Mg	+	Embossed dark red color
3.	Saponins	Aquades	-	No stable foam formation
4.	Tannins	FeCl3	+	There is a discoloration to greenish-brown
5.	Triterpenoids/Steroids	Concentrated H2SO4	+	There is a discoloration become red-purple
6.	Phenolic	FeCl3	+	There is a discoloration to blackish green

Information:

\+ : contains a group of compounds

\- : does not contain a group of compounds

Anti-Inflammatory Activity Test Results of Pink Chrysanthemum Ethanol Extract with Red Blood Cell Membrane Stabilization Method

The anti-inflammatory activity of pink chrysanthemum ethanol extract was tested with various concentration variations, namely 50 ppm, 100 ppm, 200 ppm, 400 ppm, and 800 ppm using the red blood cell membrane stabilization method. This measurement was carried out by three repetitions using a UV-Vis spectrophotometer with a wavelength of 576 nm. Stabilization of red blood cell membranes has been used as a method to determine anti-inflammatory activity in vitro.

Table 2. Results of Measurement of Absorbance Value of Pink Chrysanthemum Extract by Red Blood Cell Membrane Stabilization Method

Sample	Concentration (ppm)	Absorbance	Average	%Stabilization
Pink Chrysanthemum Flower Extract	50 ppm	0,749	0,747	15,8784
		0,752		
		0,74		
	100 ppm	0,464	0,484	45,4204
		0,451		
		0,539		
	200 ppm	0,46	0,456	48,6111
		0,456		
		0,453		
	400 ppm	0,458	0,432	51,3514
		0,419		
		0,419		
	800 ppm	0,361	0,36	59,4595
		0,361		
		0,358		

Based on the measurement results, it can be seen that the greater the concentration of the test solution, the lower the absorbance value and the higher the percent of stabilization which indicates the presence of anti-inflammatory activity that is able to protect the red blood cell membrane from damage.

Results of IC50 Analysis of Pink Chrysanthemum Flower Extract (Red Blood Cell Membrane Stabilization Method)

1. IC50 Pink Chrysanthemum Flower Extract

The results of the anti-inflammatory activity test of ethanol extract of pink chrysanthemum flowers with the red blood cell membrane stabilization method obtained the absorbance value used to obtain the percent stabilization value. The linear regression equation is obtained by plotting the concentration of the test solution with percent stability, where the concentration is as the abscess and the percent absorbance is the ordinate. The equation obtained is $y = 0.0395x + 31.944$ with a correlation coefficient value of 0.527.

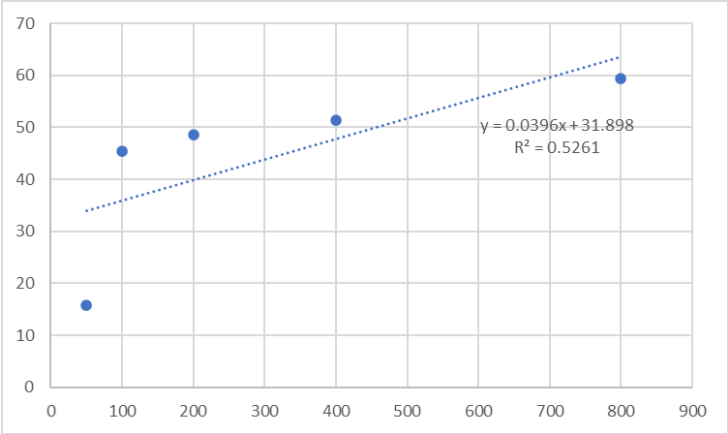

Figure 1. Graph of Anti-Inflammatory Activity Test Results of Pink Chrysanthemum Flower Extract

In the linear regression equation y = ax + b, y is the % of anti-inflammatory activity, a is the slope setting, b is the intercept setting, and x is the concentration of the test solution. The IC50 value of pink chrysanthemum flower extract was obtained through a linear regression equation, with the y variable being 50 and the x variable being the IC50 value which is interpreted as the sum of the concentration of the solution that evaluates and compares the effectiveness of an extract in inhibiting hemolysis. Anti-inflammatory activity is categorized as very active if the IC_{50} value is less than 50 µg/mL, active if it is in the range of 50-100 µg/mL, medium if it is in the range of 101-250 µg/mL, weak if it is in the range of 251-500 µg/mL, and inactive if the IC_{50} value is greater than 500 µg/mL.

The following is the calculation of the IC50 value of ethanol extract of pink chrysanthemums:

$$y = ax + b$$
$$y = 0.0396x + 31.898$$
$$50 = 0.0396x + 31,898$$
$$x = (50\text{-}31,898)/0.0396$$
$$x = 457.121212 \text{ ppm}$$

Based on these calculations, the IC50 value of ethanol extract of pink chrysanthemums of 457.121212 ppm is included in the weak category.

2. IC50 Na Diclofenak

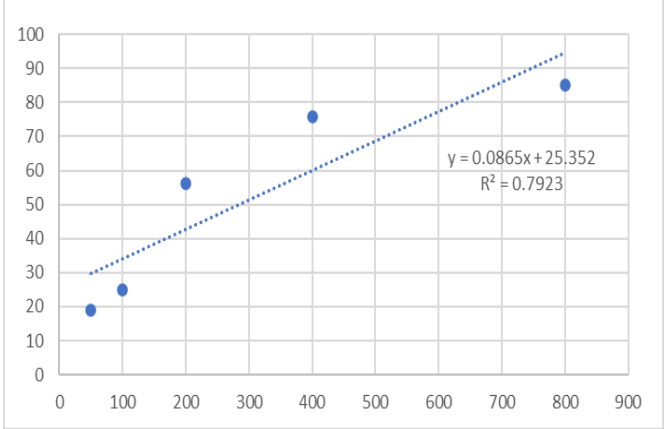

Figure 2. Graph of Anti-Inflammatory Activity Test Results of Diclofenac Sodium Solution

The equation obtained is y = 0.062x + 44.477 with a correlation coefficient value of 0.7923. The following is the calculation of the IC50 value of diclofenac sodium solution:

$$y = ax + b$$
$$50 = 0.0865x + 25,352$$
$$x = (50-25.352)/0.0865$$
$$x = 284.947977$$

From the two experiments, quite good results were obtained, with an increase in the stabilization percentage as the concentration of the test solution increased. The IC50 value obtained for chrysanthemums is 5. Pink is 457,121, while for positive control (sodium diclofenac) is 284,947. These results show that both test solutions have potential anti-inflammatory activity.

Results of Statistical Data Analysis

Table 3. Kruskal-Wallis Test Results

Solution	Concentration (ppm)	p-value	Interpretation
Pink chrysanthemum flower ethanol extract	50	0,001	There are differences between groups
	100		
	200		
	400		
	800		
Diclofenac Sodium	50		
	100		
	200		
	400		
	800		

Table 3 shows the results of the Kruskal-Wallis test on pink chrysanthemum flower extract and diclofenac sodium at five different concentrations. The results of the analysis showed a p-value of 0.001, which indicated that there was a significant difference between pink chrysanthemum flower extract and diclofenac sodium in all concentrations tested.

Table 4. Difference in Test Group Values

	BP50	BP100	BP200	BP400	BP800	KP50	KP100	KP200	KP400	KP800
BP50		0,138	0,086	0,070	0,003	0,676	0,404	0,012	0,001	0,000
BP100	0,138		0,817	0,745	0,150	0,286	0,516	0,307	0,063	0,023
BP200	0,086	0,817		0,926	0,228	0,194	0,378	0,430	0,104	0,041
BP400	0,070	0,745	0,926		0,265	0,164	0,330	0,486	0,126	0,051
BP800	0,003	0,150	0,228	0,265		0,012	0,037	0,676	0,676	0,404
KP50	0,676	0,286	0,194	0,164	0,012		0,676	0,037	0,003	0,001
KP100	0,404	0,516	0,378	0,330	0,037	0,676		0,095	0,012	0,003
KP200	0,012	0,307	0,430	0,486	0,676	0,037	0,095		0,404	0,210

KP400	0,001	0,063	0,104	0,126	0,676	0,003	0,012	0,404		0,676
KP800	0,000	0,023	0,041	0,051	0,404	0,001	0,003	0,210	0,676	

Table 4 shows the results of the Post Hoc Test using the Dunn's Test to find out the groups that have significant differences. The group that had the most difference from the other group was the 800 ppm positive control. This group has significant differences with the other 5 groups. The 800 ppm positive control was different from the group with the extract group at 50 ppm, 100 ppm, 200 ppm and with the positive control group at 50 ppm and 100 ppm. The group that had the least difference from the other groups was the 100 ppm and 200 ppm extract groups. These two groups have significant differences with 1 other group. The two groups of 100 ppm and 200 ppm extracts both had differences with the positive control group at 800 ppm.

Discussion

Pink chrysanthemums are taken as samples and go through a series of processes, from drying to extraction, to producing a viscous extract that will be tested for its anti-inflammatory activity. This extraction process aims to separate the compounds contained in the plant from the mixture or simplisia. The extraction method chosen is the Microwave Assisted Extraction method because it has the ability to speed up the extraction process so that it takes a shorter time to get optimal results when compared to conventional methods. This extraction method produces good extract quality even though it has a simpler and easier process (Damayanti et al., 2021).

In the extraction process, a thick extract of 14.805 grams was obtained with a yield of 29.61%. The greater the yield value, the better the value of the extract produced. Higher yield values indicate that more dissolved compounds are successfully extracted. High yields generally reflect the success of extraction methods in obtaining active ingredients efficiently (Yulianti et al., 2021). Many factors affect the yield of a viscous extract, including the type of solvent, extraction time, temperature, particle size of the material, type and source of the material, extraction method, and concentration of solvent and material (Senduk et al., 2020).

Based on the results of phytochemical screening, it was obtained that pink chrysanthemum flower ethanol extract contains active compounds such as alkaloids, flavonoids, tannins, steroids/triterpenoids, and phenolates where these compounds are able to act as anti-inflammatory. This result is in line with research conducted by Belhachat et al., (2023) that the results of phytochemical screening of chrysanthemum flower extract contain alkaloid compounds, flavonoids, triterpenoids, and tannins. Pink chrysanthemum ethanol extract has anti-inflammatory activity because there are compounds that play a role in reducing and relieving inflammation by inhibiting inflammatory mediators (Liu et al., 2018).

The phenol test showed positive results because a blackish-green color was formed. Phenols are one of the main compounds that act as anti-inflammatory. Phenols make an important contribution to anti-inflammatory activity through inflammatory mediator inhibition mechanisms and antioxidant activity that donates hydrogen atoms, so that free radicals are reduced to a more stable form. In other words, it can reduce inflammation caused by oxidative stress (Lumintang et al., 2022).

The flavonoid test showed positive results because there was a brick red/dark red color. One of the functions of flavonoids is to help regulate the immune response by suppressing excessive activity in immune cells such as macrophages and T lymphocytes (Fitri & Putra, 2021). The tannin test showed a positive result because there was a greenish-brown color. Tannin compounds have an OH group whose hydrogen atoms can be donated to free radicals so that they become more stable forms thereby reducing oxidative stress that contributes to the inflammatory process (Novika et al., 2021).

The steroid and triterpenoid tests showed positive results because there was a purplish-red color. Steroids/triterpenoids can inhibit the production of inflammatory mediators such as TNF-α and IL-6. These compounds can lower levels of pro-inflammatory cytokines in experimental models, thereby helping to relieve inflammatory symptoms (Rezeki et al., 2023). The alkaloid test showed positive results because there were orange deposits in the Dragendorff reagent and brown precipitates in the Wagner reagent. However, in the Mayer reagent there is no white deposit. Alkaloids can modulate immune system activity, lowering hyperactive responses that trigger inflammation (Anggarani et al., 2023).

The anti-inflammatory activity test uses the red blood cell membrane stabilization method. In the red blood cell membrane stabilization method, it is used to evaluate anti-inflammatory activity in vitro because the red blood cell membrane has a structural similarity to the lysosomal membrane. Lysosomal membranes play an important role in inflammation, and their stabilization can prevent the release of enzymes from within the lysosomes during the inflammatory process. The rupture of the lysosomal membrane will release the enzyme phospholipase, which then converts the phospholipid into arachidonic acid. This acid further triggers the formation of prostaglandins, which act as mediators in the inflammatory process (Wahdaniah et al., 2023). The release of this enzyme, which often occurs due to neutrophil activation, can cause tissue damage associated with acute and chronic inflammation (Saepudin et al., 2024).

In the anti-inflammatory test, five main solutions are used, namely isosaline solution, hyposaline, phosphate buffer, red blood cell suspension, and test solution. The results show that the negative control absorbance value is 0.888. Meanwhile, the positive control absorbance for concentrations of 50 to 800 ppm consecutively was 0.721; 0,667; 0,388; 0,215; and 0.133. For the test solution with pink chrysanthemum flower extract, the absorbance value obtained consecutively was 0.747; 0,484; 0,456; 0,432; and 0.36. These results show that the absorbance value of the test solution is quite good, with a consistent increase in effectiveness along with increasing concentration.

The anti-inflammatory activity of the ethanol extract of pink chrysanthemum flowers was shown in the decrease in absorbance value in the test solution mixture. A decrease in absorbance signals the occurrence of lower hemolysis, which reflects a higher anti-inflammatory activity of the sample (Wasiaturrahmah & Amalia, 2023). The absorbance of the solution was measured at a wavelength of 576 nm to detect hemoglobin released by lysis of red blood cells. Diclofenac sodium is used as a positive control because it is a nonsteroidal anti-inflammatory drug (NSAID) that works by inhibiting prostgland synthesis (Dr . Apt . Diana Laila Ramatillah, 2020). This mechanism involves inhibiting the enzyme

cyclooxygenase (COX) responsible for the synthesis of prostaglandins, compounds that play a role in the inflammatory process. In addition, diclofenac sodium was chosen because of its widespread use as an anti-inflammatory agent and its easy availability (Rodwell et al., 2015).

The results showed that the ethanol extract of pink chrysanthemum flowers at a concentration of 800 ppm had the highest ability in stabilizing red blood cell membranes with a stabilization rate of 59.459%. In contrast, the lowest concentration, which is 50 ppm, only produces a stabilization of 15.878%. These findings indicate that the increase in extract concentration is directly proportional to the ability of red blood cell membranes to stabilize. The higher the concentration, the greater the protection it provides against the red blood cell membrane, which also reflects the greater potential for anti-inflammatory activity. This analysis is also supported by statistical testing. The analyzed data included the stability of red blood cell membranes in diclofenac sodium solution and pink chrysanthemum ethanol extract with concentrations of 50, 100, 200, 400, and 800 ppm.

After obtaining absorbance data from UV-Vis spectrophotometry, calculations were carried out to obtain the stabilization percentage (%stability) and IC50 value. For positive control (sodium diclofenac), the percentage stabilization of concentrations of 50 to 800 ppm consecutively was 18.768%; 24,811%; 56,230%; 75,712%; and 84.984%. As for pink chrysanthemum flower extract, the value of the consecutive stabilization percentage at the same concentration was 15.878%; 45,420%; 48,611%; 51,351%; and 59.459%. This result is considered good if the % stabilization value of the pink chrysanthemum flower extract approaches or even exceeds the value produced by the positive control, in this case sodium diclofenac, which is used as the standard anti-inflammatory drug. This indicates the potential for competitive anti-inflammatory activity of pink chrysanthemum flower extract.

After obtaining the stabilization %, the IC50 value was calculated to determine the stabilization ability of the membrane against lysis at a concentration of 50%. The results showed that the IC50 value for the positive control (sodium diclofenac) was 284.947977, while for the pink chrysanthemum flower extract was 457.121212. The IC50 value of pink chrysanthemum flower extract indicates the presence of anti-inflammatory activity. However, this difference in IC50 values indicates that the anti-inflammatory activity of pink chrysanthemums is weaker compared to the positive control of sodium dichlorofenac.

The results of the analysis from SPSS using the normality test showed that the data was not normally distributed, with a significance value of 3 out of 5 concentrations greater than 0.05. Based on these results, the next step is to use the Kruskal-Wallis non-parametric test, because the data distribution is abnormal. The Kruskal-Wallis test produced an *asymptotic significance* value of 0.011, which indicates a significant difference between the concentrations tested. Therefore, this test can be continued with the Post-Hoc test using the Dunn-Bonferroni method.

The results of the Post-Hoc test showed a significance value of less than 0.05, which indicates that there is a significant difference between the concentrations tested. Thus this indicates that there is a significant difference between the concentrations of each test solution.

CONCLUSION

This study shows that the ethanol extract of pink chrysanthemum flower (Chrysanthemum morifolium) contains a variety of active compounds, including alkaloids, flavonoids, tannins, steroids/triterpenoids, and phenolates, which have the potential as anti-inflammatory agents. Based on testing using the erythrocyte membrane stabilization method, the anti-inflammatory activity of the extract increased along with the increase in concentration. The highest concentration tested, which was 800 ppm, showed the highest level of red blood cell membrane stabilization of 59.459%. However, the IC50 value obtained at 457.12 ppm indicates that the ethanol extract of pink chrysanthemum flower has anti-inflammatory activity in the weak category. These findings indicate that pink chrysanthemums may be an alternative source of natural anti-inflammatory compounds, although more research is needed to improve their effectiveness and optimize their potency compared to positive controls such as sodium diclofenac.

REFERENCE

Adiyasa, M. R., & Meiyanti, M. (2021). Utilization of traditional medicines in Indonesia: distribution and influential demographic factors. *Journal of Biomedicine and Health*, *4*(3), 130–138.

Akrom, T. H. (2021). *Immunopharmacologist Radang*. Azkiya Publishing.

Anggarani, A. M., Ilmiah, M., & Nasyaya Mahfudhah, D. (2023). Antioxidant Activity of Several Types of Onions and Its Potensial as Health Supplements. *Indonesian Journal of Chemical Science*, *12*(1), 103–111.

Arfania, M., Frianto, D., Mudrikah, S., & Amelia, T. (2023). Literature Review The Role of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) in Analgesia for Postoperative Pain Management. *Innovative: Journal Of Social Science Research*, *3*(2), 263–274.

Arida, A., Protogerou, A. D., Kitas, G. D., & Sfikakis, P. P. (2018). Systemic inflammatory response and atherosclerosis: the paradigm of chronic inflammatory rheumatic diseases. *International Journal of Molecular Sciences*, *19*(7), 1890.

Belhachat, D., Tidjani, R., Makhlouf, A., & Balhachat, M. (2023). Phytochemical Screening, Composition and In Vitro Antioxidant Activity of Algerian Chrysanthemum coronarium (L.) Extracts. *South Asian Journal of Experimental Biology*, *13*(3).

Damayanti, A., Buchori, L., & Sulardjaka. (2021). Anthocyanin extraction of red dadap flowers using the Mae (Microwave Assisted Extraction) method. *Indonesian Journal of Halal*, *3*(2), 100–105.

Dr . Apt . Diana Laila Ramatillah, M. . F. (2020). *Drug Interaction Book*. 1–67.

Fitri, W. E., & Putra, A. (2021). Review: The Role of Flavonoid Compounds in Boosting the Immune System During the Covid-19 Pandemic. *Syedza Saint National Seminar*, 61–72.

Hendayana, H. (2023). North Minahasa Towards Stunting-Free. *Administrative Pulpit Of FISIP UNTAG Semarang*, *20*(2), 329–348.

Liu, Y. H., Mou, X., Zhou, D. Y., Zhou, D. Y., & Shou, C. M. (2018). Extraction of flavonoids from Chrysanthemum morifolium and antitumor activity in vitro. *Experimental and*

Therapeutic Medicine, *15*(2), 1203–1210. https://doi.org/10.3892/etm.2017.5574

Lumintang, V. S., Wowor, K. B., Wowor, K. B., Pangemanan, K., Mandagi Jr, S. C., Kalalo, M. J., & Antasionasti, I. (2022). Innovation of Sunscreen Lotion from Chrysanthemums as a Leading Health Cosmetic of Tomohon City. *Journal of Regional Policy and Innovation*, *1*(2), 13–17. https://doi.org/10.56585/jkdid.v1i2.13

Luthfianto, D., Indriputri, C., AK, S. T., Imun, M., Kp, D. R. P. S., Faizal, I. A., AK, S. T., Imun, M., Husen, F., & Supriatin, T. (2023). *Textbook of Immunology*. Pangkalpinang: Science Techno Direct.

Novika, D. S., Ahsanunnisa, R., & Yani, D. F. (2021). Anti-Inflammatory Activity Test of Ethanol Extract of Star Fruit Leaf (Averrhoa bilimbi L.) Against the inhibition of protein denaturation. *Stannum : Journal of Science and Applied Chemistry*, *3*(1), 16–22. https://doi.org/10.33019/jstk.v3i1.2117

Prabawati, N. B., Oktavirina, V., Palma, M., & Setyaningsih, W. (2021). Edible flowers: Antioxidant compounds and their functional properties. *Horticulturae*, *7*(4), 66.

Prince, M., Wimo, A., Guerchet, M., Ali, G.-C., Wu, Y.-T., & Prina, M. (2015). *World Alzheimer Report 2015. The Global Impact of Dementia: An analysis of prevalence, incidence, cost and trends.* Alzheimer's Disease International.

Rezeki, T. R., Nasution, H. M., Nasution, M. P., & Rahayu, Y. P. (2023). Phytochemical screening and isolation of steroid/triterpenoid compounds from n-hexane extract of jackfruit seeds (Artocarpus heterophyllus Lam). *Journal of Pharmaceutical and Sciences*, *6*(4), 1854–1861. https://doi.org/10.36490/journal-jps.com.v6i4.331

Rodwell, V. W., Bender, D. A., Botham, K. M., Kennelly, P. J., & Weil, A. P. (2015). Harper's Illustrated Biochemistry, 30th Ed. In *Harper's Illustrated Biochemistry*.

Ross, E. A., Devitt, A., & Johnson, J. R. (2021). Macrophages: the good, the bad, and the gluttony. *Frontiers in Immunology*, *12*, 708186.

Saepudin, S., Hidayat, T. S., Al-Azzahra, Y., & Cahyati, A. (2024). Anti-Inflammatory Activity Test of Yellow Root Stem Extract (Arcangelisia flava (L.) Merr.) Secara In Vitro. *Jurnal Buana Farma*, *4*(1), 1–10. https://doi.org/10.36805/jbf.v4i1.934

Senduk, T. W., Montolalu, L. A. D. Y., & Dotulong, V. (2020). The rendement of boiled water extract of mature leaves of mangrove Sonneratia alba. *Jurnal Perikanan Dan Kelautan Tropis*, *11*(1), 9. https://doi.org/10.35800/jpkt.11.1.2020.28659

Suryandari, S. S., De Queljoe, E., & Datu, O. S. (2021). Anti-inflammatory activity test of sesewanua leaf ethanol extract (Clerodendrum squamatum Vahl.) on carrageenan-induced white rats (Rattus norvegicus L.). *Pharmacon*, *10*(3), 1025–1032.

Wahdaniah, W., Sabrina Azani, A., & Kamilla, L. (2023). Anti-Inflammatory Activity Test of Jackfruit Seed Ethanol Extract (Artocarpus heterophyllus Lam.) against the stabilization of red blood cell membranes. *Journal of the Equator Laboratorywa*, *7*(1), 102. https://doi.org/10.30602/jlk.v7i1.1254

Wasiaturrahmah, Y., & Amalia, N. (2023). Anti-inflammatory Potential of Sentul Lyre Leaf Extract (Sandoricum koetjape Merr) by Red Blood Cell Membrane Stabilization Method. *Ibn Sina Scientific Journal (JIIS): Pharmaceutical and Health Sciences*, *8*(1), 125–133. https://doi.org/10.36387/jiis.v8i1.1277

Yuan, H., Jiang, S., Liu, Y., Daniyal, M., Jian, Y., Peng, C., Shen, J., Liu, S., & Wang, W. (2020). The flower head of Chrysanthemum morifolium Ramat.(Juhua): A paradigm of flowers serving as Chinese dietary herbal medicine. *Journal of Ethnopharmacology, 261*, 113043.

Yulianti, W., Ayuningtyas, G., Martini, R., & Resmeiliana, I. (2021). Effect of Extraction Method and Solvent Polarity on Total Phenolic Levels of Kersen Leaves (Muntingia calabura L). *Journal of Applied Science, 10*(2), 41–49. https://doi.org/10.29244/jstsv.10.2.41-49