http://ejournal.seaninstitute.or.id/index.php/healt
Jurnal eduhealth, Volume 14, No 02, 2023
E-ISSN. 2808-4608

Anti-inflammatory Activity of Taro Stem Ethanol Extract (*Colocasia esculenta* (L.) Schott) In Vitro

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ARTICLE INFO

Keywords:
anti-inflammatory,
taro stem,
in vitro.

ABSTRACT

Inflammation is a complex response of vascular tissue to hazards such as pathogens, irritants, and damaged cells/tissues. Inflammation is caused by the release of chemical mediators from damaged tissue and cell migration. The purpose of this study was to determine the efficacy of ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) which is used as an anti-inflammatory agent and to determine the concentration of ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) which can provide anti-inflammatory activity. The method used in this research is the stability of the red blood cell membrane. The concentrations used were 0 ppm, 25 ppm, 75 ppm, 150 ppm and 100 ppm. The results of the percentage inhibition of hemolysis stability of the red blood cell membrane of taro stem extract concentrations of 0 ppm (00.00%), 25 ppm (82.58%), 75 ppm (83.47%), 150 ppm (47.17%), 100 ppm (24.23%). Testing the anti-inflammatory activity of the ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) using the red blood cell membrane stability method has efficacy as an anti-inflammatory agent. The concentration of 25 ppm (82.58%) has the highest anti-inflammatory activity and with a small concentration of extract can produce great anti-inflammatory activity, compared to 100 ppm aspirin as a positive control of 24.23%. Testing the anti-inflammatory activity of the ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) using the red blood cell membrane stability method has efficacy as an anti-inflammatory agent. The concentration of 25 ppm (82.58%) has the highest anti-inflammatory activity and with a small concentration of extract can produce great anti-inflammatory activity, compared to 100 ppm aspirin as a positive control of 24.23%. Testing the anti-inflammatory activity of the ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) using the red blood cell membrane stability method has efficacy as an anti-inflammatory agent. The concentration of 25 ppm (82.58%) has the highest anti-inflammatory activity and with a small concentration of extract can produce great anti-inflammatory activity, compared to 100 ppm aspirin as a positive control of 24.23%.

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1. INTRODUCTION

Inflammation is a complex response of vascular tissue to hazards such as pathogens, irritants, and damaged cells/tissues[1]. People use synthetic drugs or use traditional medicines. Synthetic drugs commonly used to relieve inflammation are Non-Steroid Anti-Inflammatory Drugs (NSAIDs) such as ibuprofen, aspirin and mefenamic acid and others. While the traditional medicines commonly used are turmeric, kencur, tapak liman, purple sweet potato, suji leaves, cashew and also black cumin[2].

Steroid and Non-Steroid Anti-Inflammatory Drugs (NSAIDs) are drugs indicated to treat inflammation and pain both orally and topically. However, prolonged use can result in side effects such as stomach ulcers, impaired kidney function and hormone disturbances. In addition, the use of anti-inflammatory drugs when consumed in the long term can reduce the function of organs such as the kidneys, liver, digestive organs and cardiotoxic. Alternative treatment as an effort to reduce the risk of side effects of these drugs can use traditional medicines as therapy, both orally and topically[2]. One of the plants that can be used as traditional medicine is the taro plant.

The taro plant comes from the Colocasia genus which belongs to the Araceae family, which is a tuber plant that can be used as food. Taro plants have stems, which are thought to function as new wound dressings or as an alternative to wound medicine[3]. Taro stem (*Colocasia esculenta* (L.) Schott) contains secondary metabolites including flavonoids, tannins, alkaloids, saponins, steroids and terpenoids. The phytochemical content contained in the stems of taro (*Colocasia esculenta* (L.) Schott) can help heal wounds. Flavanoids, alkaloids, tannins, saponins, steroids, and terpenoids have antibacterial functions. In addition, flavonoids also function as anti-inflammatory and antioxidants. Meanwhile, tannins function as astringents which can cause skin pores to narrow and stop light bleeding[4]. Based on the chemical content contained in taro stem which is suspected of having potential as an alternative wound medicine, research on the Anti-inflammatory Activity of Taro Stem Ethanol Extract (*Colocasia esculenta* (L.) Schott) as an anti-inflammatory agent in vitro needs to be carried out. The Research purposes are to know the efficacy of ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) which is used as an anti-inflammatory agent, and to know the concentration of ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) which can provide anti-inflammatory activity effect

2. METHOD

This type of research and research design is descriptive experimental research. This research was conducted in November 2022-June 2023 at the Ibnu Sina Ajibarang Institute,district Ajibarang. Regency. Banyumas. The population in this study were all taro stems which can be used as anti-inflammatory agents in Pandansari Village, Kec. Ajibarang. Regency. Banyumas. The sample used was linjik taro stem (*Colocasia esculenta* (L.) Schott) obtained in Pandansari Village, Kec. Ajibarang, Kab. Banyumas. Inclusion Criteriab are Colocasia esculenta(L.) Schott, Plant age 5-6 months, Consumable, White wistar rats with a body weight of 250-300 grams, Age 6-8 weeks.

3. RESULTS AND DISCUSSION

This study aims to determine the efficacy of ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) which is used as an anti-inflammatory agent and to determine the concentration of ethanol extract of taro stem (*Colocasia esculenta* (L.) Schott) which can provide anti-inflammatory activity. This research was conducted with eleven stages of testing. This stage includes: sampling, plant determination and ethical clearance, simplicia production, preparation of ethanol extract of taro stems using the maceration method, standardization of ethanol extract of taro stems, preparation of the required solution, preparation of concentrations of ethanol extract of taro stems and aspirin, preparation of red blood cell suspension white wistar rats, anti-inflammatory activity test, UV-Vis spectrophotometry, percentage (%) hemolysis inhibition calculation.

1. The results of determining the taro plant at the Environmental Laboratory, Faculty of Biology, Jenderal Soedirman University Purwokerto, showed that the plant used in this study was the taro plant (*Colocasia esculenta* (L.) Schott) belonging to the Araceae family with Certificate Number B/850/UN.23.6 .10/TA.00.01/2022. Results of ethical clearance at the Muhammadiyah University Purwokerto Health Research Ethics Commission with Registration Number KEPK/UMP/22/V/2023.
2. A total of 100 grams of taro stem simplicia was extracted by maceration using 70% ethanol solvent with the yield of simplicia and the yield of ethanol extract of taro stems can be seen in the following table:

Table 1. Yield of Taro Stem Simplicia

Sample	Dry simplicia weight (g)	Wet simplicia weight (g)	yield (%)
taro stem	102.05	1938,86	5,26

Table 2 Yield of Taro Stem Ethanol Extract

Sample	Powder Weight (g)	Extract Weight (g)	yield (%)
taro stem	100	26,71	26,71

3. Extract Standardization

Standardization is a process that guarantees that the final drug product (drug, extract or extract product) has certain parameter values that are constant and determined in advance[5].Extract standardization has two parameters, namely specific and non-specific parameters.

a) Extract identity is a preliminary test that is used as an initial introduction and the part of the plant to be used[6]. This research is in accordance with the results of the determination, namely taro stems with scientific names*Colocasia esculenta*(L.) Schott family Araceae. The part of the plant used is the stem.
b) The organoleptic test of taro stems was a viscous extract with a blackish brown color and a characteristic odor of taro stems.
c) Determination of the levels of water-soluble compounds and ethanol aims to determine the amount of compounds contained in water (polar) and ethanol (semi-polar-non-polar). The results obtained were that the water soluble compound content in taro stems was 0% and the ethanol soluble compound content was 20%. The sum of the results of the content of water-soluble compounds and ethanol meets the requirements, namely not exceeding 100%. The sum of the levels of water-soluble compounds and ethanol in an extract should not be more than 100%. Based on the results obtained, the extract is more dissolved in ethanol than water. This means that the active compounds in the extract are more likely to be easily extracted in ethanol than water. Because ethanol is a universal solvent, it can attract polar and non-polar compounds. While water only attracts polar compounds[7].
d) Determination of water content aims to provide a minimum limit or range regarding the amount of water content in the material (extract) the higher the water content, the easier it is for fungi and mold to grow. The results of the water content test met the requirements, namely 0.71%. According to FHI (2000), generally the required moisture content is less than 10%[6]
e) Determination of drying shrinkage in extracts is one of the parameters that must be met because it can provide a maximum limit (range) of the amount of compounds lost in the drying process. In this drying shrinkage test, the remaining substance was measured after drying at 105°C for 30 minutes. At 105°C water will evaporate and compounds that have a lower boiling point than water will also evaporate. The results of determining the dry shrinkage of dry extract of taro stems were 0.47%. There are no conditions or allowable ranges for the drying shrinkage parameter[6].The smaller the value for determining dry shrinkage, the better the drying process will be carried out on the sample. This means the smaller the water content in the extract, so it can reduce the possibility of fungus growing[8].
f) The specific gravity parameter is the mass per unit volume at room temperature 25ºC using a special pycnometer tool aimed at determining the specific gravity of the extract, namely providing a limit on the magnitude of the unit volume mass which is a special parameter for liquid extracts to concentrated (thick) extracts which can still be calculated[5].The specific gravity test is carried out using a pycnometer by first cleaning the pycnometer and drying it until there is no water in it at all. It aims to obtain the empty weight of the tool. If there is still water in it, it will affect the results obtained[5].The specific gravity of the extract obtained was 1.083 gr/ml.Specific gravity values that are closer to 1 or more than 1 indicate that the extract is more miscible with water, and vice versa. The specific gravity value obtained can be said to be more than 1 so that the extract can mix with water[9].

Table 3 Specific Parameters of Taro Stem Extract

No.	Parameter	Results
1	Extract identity	Extract name: taro stem extract Latin name: *Colocasia esculenta*(L.) Schott Plant part: stem
2	Organoleptic	Viscous, blackish brown, characteristic odor
3	Water soluble compound content	0%
4	Levels of ethanol soluble compounds	20%
5	Identification of chemical content	flavonoids, tannins, alkaloids, saponins, steroids.

Table 4 Non-Specific Parameters of Taro Stem Extract

No.	Testing	Results
1	Drying shrinkage	0.47%
2	Water content	0.71%
3	Specific gravity	1.0183 gr/ml

4. Anti-inflammatory Activity Test

The anti-inflammatory activity test used in this study was an in vitro test using the red blood cell membrane stabilization method. In vitro test using the methodErythrocyte membrane stabilization is easy to obtain, easy to isolate from blood, has the same cell membrane structure as lysosomes, so this method can be used as a test to determine anti-inflammatory activity.. Using the in vitro method, because it is fast, can be standardized and the process can be controlled so as to get accurate results besides that in this method only uses blood samples, so it does not require more treatment in rats meaning that after the blood is taken the rats are no longer used . However, the in vitro method requires accuracy so as to produce accurate results. In addition, in vitro tests require special tools such as spectrophotometry.

Testing the anti-inflammatory activity of the ethanol extract of taro stems 25 ppm, 75 ppm, 150 ppm produced an average percentage of inhibition that was better than aspirin 100 ppm, which can be seen in table 5:

Table 5 Percentage (%) of Taro Stem Ethanol Extract Inhibition

Concentration (ppm)	Mean % Inhibition ± SD
negative control	00.00 ± 0.00
25	82.58 ± 1.74
75	83.47 ± 0.62
150	47.17 ± 4.21
Positive control (aspirin)	24.23 ± 1.98

Test samples that have anti-inflammatory activity can be seen from the decrease in hemoglobin absorbance detected in the test solution. The smaller the absorbance value, the red blood cells will be stable, experience less lysis and have a large percentage of hemolysis inhibition. Likewise vice versa if the greater the absorbance value, the blood cells become unstable, experience lysis and have a small percentage of inhibition[10]. Lysis can occur due to the induction of hyposaline solutions which cause oxidative stress and disrupt membrane stability[11]. The relationship between inhibition and inflammation is that inhibition of the ethanol extract of taro stems can inhibit or inhibit the formation of cyclooxygenase-1 and cyclooxygenase-2 enzymes. So inflammation will be reduced [12].

At concentrations of 25 ppm and 75 ppm there was an increase in inhibition of hemolysis, but when the concentration was increased to 150 ppm there was a decrease in activity. This happens because the extract is at its saturation point[13]. Aspirin has a higher absorbance so it will produce a small percentage of inhibition[14]. The greater the absorbance produced, the red blood cell membrane will be increasingly unstable and can experience lysis[10].

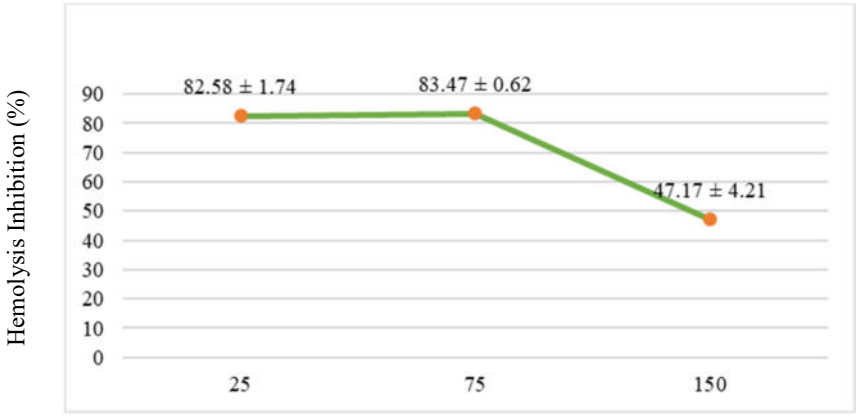

Figure 1. Hemolysis Inhibition

5. Data analysis

The results of data analysis on the anti-inflammatory activity of ethanol extract of taro stems using the normality test showed that all data were normally distributed, where the significance value was p>0.05.

Table 6 Data Analysis Results of Shapiro Wilk Normality Test

Sample	Significance Value
F0	0.000
F1	0.326
F2	0.468
F3	0.407
F4	0.140

Then proceed with the Levene test of homogeneity test can be seen in table 4.7. If the significance value of p> 0.05, it can be said to be homogeneous and vice versa. The homogeneity test results showed a homogeneous significance value of 0.009 and continued with the One Way ANOVA parametric analysis which showed a significance value of 0.000 (p <0.05).

Table *7* Results of Data Analysis of Homogeneity Test and ANOVA

Test	Significance Value
Homogeneity	0.009
ANOVA	0.000

The results of the Post Hoc LSD (Fisher Least Significant Difference) test showed a significant difference in the mean between groups of 0.000 (p <0.05), meaning that there was a significant difference. While the concentrations of 25 ppm and 75 ppm showed no significant difference.

Table 8 Results of LSD Post Hoc Test Data Analysis

Concentration	F0	F1	F2	F3	F4
F0	-	0.000*	0.000*	0.000*	0.000*
F1	0.000*	-	0.636	0.000*	0.000*
F2	0.000*	0.636	-	0.000*	0.000*
F3	0.000*	0.000*	0.000*	-	0.000*
F4	0.000*	0.000*	0.000*	0.000*	-

*Post-hoc LSD**significantly different (p<0.05)

From this test, all groups in the concentration series of taro stem ethanol extract had a mean

percentage (%) of hemolysis inhibition that was greater than the positive and negative groups. So this shows that the ethanol extract of taro stems has a better anti-inflammatory effect than the positive and negative controls, even at the lowest concentration.The concentration of 25 ppm was chosen because at this concentration it has the highest anti-inflammatory activity of 82.58% and with a small concentration of extract it produces great anti-inflammatory activityso that this concentration can be used as an anti-inflammatory agent[15].

Active compounds that play an important role in stabilizing red blood cell membranes are flavonoids, saponins and tannins. The relationship between aspirin and the active compounds in taro stems is to inhibit prostaglandins, which are inflammatory mediators derived from the cyclooxygenase enzyme.

4. CONCLUSION

Testing of taro stem ethanol extract(Colocasia esculenta(L.) Schott) with the method of stability of the red blood cell membrane has efficacy as an anti-inflammatory agent. The concentration of 25 ppm of 82.58% has the highest anti-inflammatory activity and with a small concentration of extracts can produce great anti-inflammatory activity, compared to 100 ppm aspirin as a positive control of 24.23%.

ACKNOWLEDGMENT

I would like to thanks for UPPM STIKes Ibnu Sina Ajibarang for funding this research so that it can expedite the research process.

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