Activity Test of Ethanol Extract from Pulai Bark (*Alstonia scholaris L.*) Antiinflammation in Mice (*Mus musculus*)

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Abstract. Inflammation is a complex biological response of vascular tissue to noxious stimuli such as pathogens, damaged body cells, or irritants. The drugs commonly used are non-steroidal anti-inflammatory drugs. However, long-term use of non-steroidal anti-inflammatory drugs can cause ulceration and bleeding in the lower GI tract. So that alternative treatments that can be used are medicinal plants, one of which is the wood of pulai. Pulai bark (Alstonia scholaris L.) contains secondary metabolites of alkaloids, flavonoids, polyphenols, terpenoids and steroids. Flavonoids are able to provide anti-inflammatory effects. The aim of this study was to determine the anti-pain activity of the ethanol extract of the bark of the bark of pulai (Alstonia scholaris L.) on the inflammation of mice (Mus musculus). Extraction was carried out by continuous maceration method (remaceration) using 96% ethanol as solvent. Anti-pain testing on inflammation of mice using the true experimental method, mice were divided into 5 groups, namely the negative control group was given aquadest, the positive control was given 0.39 g/30-g BW of diclofenac sodium, the test group was given ethanol extract of pulai bark as much as 0.5 mL/30 g BW with a dose of 400 mg/kgBW, 200 mg/kgBW and 100 mg/kgBW. After 1 min, 1% acetic acid was induced at a dose of 10 mL/30 gBW. Observation of healing time was carried out by observing the stretching of the mice that had been given treatment, which was marked by reduced stretching of the mice. The data were analyzed statistically using the One Way Anova test. The data obtained showed that the ethanol extract of pulai bark at a dose of 400 mg/kgBW, 200 mg/kgBW and 100 mg/kgBW had an anti-pain effect on inflammation in mice. The effective dose as an anti-pain in inflammatory mice is a dose of 200 mg/kgBW.

Keywords: Inflamation · Anti-pain · Pulai bark · Mice

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1 Introduction

Inflammation is a complex biological response of vascular tissue to noxious stimuli such as pathogens, damaged cells, or irritants. Without inflammation, wounds and infections will never heal and can result in dangerous tissue damage [1].

Inflammation is one of the processes that underlie various disease due to over-response [2]. The incidence of diseases involving inflammation in the body is quite high in Indonesia. The national prevalence of diabetes mellitus ulcer disease is 1.5%, Asthma 2.4%, Acute Respiratory Infection (ARI) 4.4%, Pneumonia 2.0%, Joint Disease 7.30%, Tumor/Cancer Disease 1.73%, Hepatitis 0.39%, these diseases include diseases that have an inflammatory reaction [3].

The drugs commonly used are non-steroidal anti-inflammatory drugs. However, long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) can cause ulceration and bleeding in the lower gastrointestinal tract. It was reported that NSAIDs cause surface wounds by affecting the integrity of the gastrointestinal mucosal membrane [4].

Overcoming the side effects of these anti-inflammatory drugs can be used alternative medicine such as utilizing medicinal plants [5]. Pulai (Alstonia Scholaris L.) is a plant that is widely used in traditional medicine, especially in India and Southeast Asia, including Indonesia. Although traditionally Pulai (Alstonia sholaris L.) is used to treat various types of diseases, but reports of its bioactivity function as anti-diabetes mellitus, anticancer, antimicrobial, antioxidant and anti-inflammatory drugs [6].

Based on previous research of Arulmozhi [7] the dichlormethane (DCM) fraction of the ethanol extract of the leaves of the wood of pulai (Alstonia scholaris L) was shown to have analgesic and anti-inflammatory activity. Meanwhile, in this study, the bark of pulai was tested. Because in a previous study according to Zuraida et al. [8], the ethanol extract of the bark of the pulai bark can be used as an antioxidant. These antioxidants have various pharmacological effects, one of which is anti-inflammatory [9, 10]. In addition, the bark of pulai contains one of the secondary metabolites, namely flavonoids. Flavonoids can inhibit mediators of inflammation, namely cyclooxygenase or lipoxygenase.

Based on this description, there is no clear information regarding the pharmacological effect of pulai bark as an anti-inflammatory, so it is important to conduct research to test the anti-inflammatory activity of the ethanol extract of pulai bark (Alstonia Scholaris L.) in vivo in mice.

2 Methods

2.1 Scope

The scope of this research is in the fields of phytochemistry, pharmacognosy, traditional medicine and pharmacology. This scope also refers to the vision and mission of the D-III Pharmacy study program at the Medica Husada Mataram Polytechnic, namely developing traditional medicines.

2.2 Tools and Materials

Tools: Glassware, 1 Rotary evaporator, 1 set of vacuum rotary evaporator, 1 maceration vessel, 1 set of blender, 2 stirring rods, 3 porcelain cups, 1 set of water bath, 1 analytical balance, 5 glass beakers, 1 100 measuring cup ml, 1 spatula, 2 tissue packs, 1 mesh 40, 1 bunher funnel, 2 3-L glass jars, 5 erlenmeyer, Aluminum foil, filter paper, 2 syringes 1 ml, 2 sonde 1 mL, 2 knives, 3 mouse cages.

Ingredients: Simplicia powder from the bark of pulai wood, 4 L of ethanol 96%, Aquadest, 1 strip of Diclofenac Na, 25 mice, 0.5% acetic acid.

2.3 Data Collection Technique

The data collection technique in this study was carried out with primary data, namely direct observation or observation of the results of the anti-inflammatory activity of the ethanol extract of the bark of pulai (Alstonia scholaris L.) in mice (Mus musculus). The results of the observation of the anti-inflammatory activity are briefly shown in the table below.

2.4 Research Variable

The independent variable used in this study was the bark extract of pulai (Alstonia scholaris L.). The dependent variable used in this study was anti-inflammatory activity (pain).

2.5 Data Analysis

The data obtained from the observations and calculations of the stretching of mice were analyzed statistically using the SPSS (Statistical Product and Service Solution).

3 Result and Discussion

3.1 Results

3.1.1 Simplicia

The resulting simplicia powder is brown in color, has a bitter taste and a distinctive pungent aroma. The bark powder produced was 618.64 g and 500 g was taken for extraction.

3.1.2 Extraction

The resulting extract is a thick extract of the bark of the island with a distinctive smell and a brick red color, obtained from maceration as much as 25.86 g of 500 g of simplicia powder macerated with 3.5 L of 96% ethanol (Fig. 1).

Fig. 1. The extraction results.

3.1.3 Yield extract

$$\begin{aligned}\text{Yield extract} &= \frac{\text{Bobot Ekstrak}}{\text{Bobot Simplisia}} \times 100\% \\ &= \frac{25,86\,\text{g}}{500\,\text{g}} \times 100\% \\ &= 5,172\%\end{aligned}$$

3.1.4 Anti-inflammatory Activity Test (Pain)

Table 1. Mice stretching percentage.

No	Control	Percent of pain power
1	Negative control	0%
2	Positive control	66,09%
3	Dosage I (400 mg/kgBB)	59,39%
4	Dosage II (200 mg/kgBB)	62,49%
5	Dosage III (100 mg/kgBB)	38,81%

3.1.5 Shapiro Wilk Test Result

Table 2. Shapiro wilk rest result.

Variable	Sig. Shapiro Wilk	Z table value (5%)	Note
Pulai Bark Ethanol Extract Activity	0,052	0,05	H0 accepted

3.1.6 Homogenity Test

Table 3. Homogenity test.

Variable	Sig.	5% rate	Note
Pulai Bark Ethanol Extract Activity	0,055	0,05	H0 accepted

3.1.7 *One-Way* ANOVA the Activity Test of Ethanol Extract of Pulai Bark

Table 4. LDS test.

Source	F count	Sig.
Pulai Bark Ethanol Extract Activity	3,343	0,03

3.2 Discussion

In this study, mice (Mus musculus L.) were used, namely male mice aged 2–3 months with an average weight of 30 g. Male mice were chosen because they have a faster drug metabolism rate with more stable biological conditions than female mice. Mice were fasted for 8 h before treatment while still being given drinking water.

The first thing to do is to prepare the materials that will be used. The materials used were 2 kg of pulai bark (Alstonia scholaris L.), 3 L of 96% ethanol solvent and mice. The bark of the pulai wood used is the bark of a young stem, that is, the bark of the stem which is still hard in texture, still has sap and has not yet dried or the inside is still yellow or white. The bark of the pulai bark is then washed in running water 3 times, and if the pulai bark contains black spots then brushed first and then washed with running water. After that, the bark of the pulai is chopped, the bark of the pulai is finely chopped to make it easier during the drying process. The bark of the island is dried by arranging it in a tray and then letting it dry. Puli bark is perfectly dry $\pm$ 6 days which is indicated by if the bark of the island is broken there will be a cracking sound, the dry bark of the island is 670.17 g. Then the bark of the dried pulai logs was blended and sieved using mesh number 40.

The resulting simplicia powder from the bark of pulai bark was 618.64 g and then 500 g were weighed for remaceration. Remaceration is used because at the time of remaceration there is a solvent replacement. With this solvent replacement, several things happened, including the amount of solvent used was more so that more compounds were attracted. In addition, because it uses a new solvent, the concentration gradient between the solvent and the cell is much different, making it easier to withdraw the compounds that are in the cell. Then because remaceration is an extraction with a simple process or workmanship and equipment, besides that it is also a type of cold extraction and the content of flavonoids which have anti-inflammatory benefits (pain) is a group of compounds that cannot tolerate heat and are easily oxidized at high temperatures. This

Table 5. Pulai bark ethanol extract activity.

Dosage	1	2
Pulai Bark Ethanol Extract Activity		
K+ (Na Diklofenak)	6,280	
P2 (200 mg/kgBB)	6,946	
P1 (400 mg/kgBB)	7,522	
P3 (100 mg/kgBB)		11,334
K – (Aquadest)		18,514

remaceration uses 96% ethanol as solvent because 96% ethanol is a volatile polar solvent compound so it is good to use as an extract solvent. The extract produced from 500 g of simplicia powder that has been repressed and evaporated is 25.86 g. The extract was then transferred to a 100 cc medicinal pot and stored in the refrigerator to avoid the growth of microbes in the extract.

The test was carried out for 2 days by observing the stretching of the mice. On the first day, the mice wriggled in negative and positive controls, then on the second day, the mice wriggled that had been treated with extracts of dose I, dose II and dose III. The test was observed for 2 h 30 min. First, the mice were given control/treatment, a few minutes after that they were immediately given pain induction using 0.5% acetic acid at a dose of 10 ml/kgBW which was increased to a dose of 20 ml/kgBW because based on research Afrianti et al. (2014) used 1% acetic acid. With a dose of 10 ml/kgBW, because in this study using acetic acid with a concentration of 0.5%, the dose was increased to 2 times so that it was equivalent to 1% acetic acid. Administration of acetic acid in experimental animals is used as a pain inducer because it causes pain due to severe irritation of the mucous membrane of the abdominal cavity so that the legs are pulled back, stretched and the abdomen touches the bottom of the plate form. This type of pain includes visceral or abdominal pain that is compressive and accompanied by a vegetative reaction. This pain is caused by a stimulus that stimulates pain nerves in the visceral area, especially in the chest and abdominal cavities. Then the mice wriggled every 10 min for 2 h 30 min for each control/treatment and dose. The analysis was carried out by comparing the amount of stretching that occurred after administration of the extract dose I, dose II and dose III with Diclofenac Sodium as a positive control and aquadest as a negative control and aquadest was also used as a solvent for positive control and extract. Stretching was counted for 2 h 30 min after intraperitoneal administration of acetic acid. The average number of stretching every 10 min for 2 h 30 min can be seen in Table 1.

Before testing, it is necessary to prepare a negative control, namely aquadest, a positive control, namely 4 tablets of Diclofenac Sodium crushed and then weighed as much as 0.39 g (according to the calculation of the dose for mice) and then dissolved using 100 mL of aquadest. Then for the extract a solution was made for each dose, for the first dose the extract was weighed as much as 0.06 g and dissolved in 2.5 ml of distilled water, for the second dose the extract was weighed as much as 0.03 g and dissolved with 2.5 ml of distilled water. And for dose III the extract was weighed as much as 0.015

and dissolved in 2.5 ml of distilled water. However, because to avoid a lack of solution when treating mice and because the dose was too small so that it could not be weighed, then for the extract doses I, II and III were made 2 times or each dose for weighing the extract and the solvent was multiplied by 2. All treatments were given through orally, 0.5 mL for negative control, 0.2 mL for positive control and 0.5 mL of dose I, dose II and dose III extract.

Based on the observation of the number of stretching every 10 min, it can be seen that from 10 to 60 min most of the treatments showed the greatest stretching and decreased in the following minutes to 150 min, almost all treatments did not show any signs of pain, except negative control group. This also shows that at the 60th minute, the acetic acid used as a pain inductor has begun to weaken, except for the negative control group, which actually has the highest number of stretches at 10 to 60 min, then it rises and falls until the 150th minute. The negative control (aquadest) was the largest compared to the positive control group and the extract group. This is due to the absence of pharmacological activity of aquadest in reducing pain caused by intraperitoneal administration of acetic acid.

To determine the presence or absence of the activity of the ethanol extract of the bark of pulai (Alstonia scholaris L.) as an anti-inflammatory in mice, data analysis was carried out using the One Way Anova or one-way Anova method. Normality and homogeneity tests are a requirement to perform a one-way ANOVA test on the data used, if the data is homogeneous and normal then it may be continued to the one-way ANOVA test. The normality test (with the Shapiro-Wilk theory) based on Table 2 shows that the p_value of Shapiro Wilk is greater than the value of the significant level or 5% error. So it was decided that H0 was accepted, which means that the data on the anti-inflammatory activity (pain) of the ethanolic extract of the pulai bark followed a normal distribution pattern. Meanwhile, the homogeneity test based on Table 3 shows that the value of P_value (Sig) (0.055) is greater than the value of the significance level of 5% or 0.05 ($p < 0.05$). Thus, it was decided that there was acceptance of H0, which means the data on the anti-inflammatory activity (pain) of the ethanolic extract of the bark of the island were homogeneous.

The ANOVA test was carried out to see the extent of the difference in the effect on the treatment carried out. Based on Table 4 the values in the column Sig. Showed a significant value for data on the activity of the ethanol extract of the pulai bark. The significant value for the activity data of the ethanol extract of the pulai bark is 0.03 (smaller than the specified error, which is 5%). So that the decision taken is to reject H0 that there is a significant difference in the effect of dose differences on the activity of the ethanol extract of the pulai bark in mice.

Furthermore, further tests were carried out to determine which concentrations were different using the LSD post hoc test, based on Table 5, the results showed that for differences in concentration in the activity data of the ethanol extract of the pulai bark, it was divided into 3 groups, namely the first group K+ (Na Diclofenac), P1 (400 mg/kgBW), P2 (200 mg/kgBW) and P3 (100 mg/kgBW) and K- (Aquadest) groups. At the dose in 1 group is considered to have the same activity while between groups there is a significant difference. For the best dose of ethanol extract of pulai bark, namely at P2 with a dose of

200 mg/kgBW with the average difference with the positive control treatment K+ only 0.666.

The chemical content of the island's bark includes saponins, polyphenols and flavonoids which are useful as antioxidants, anti-inflammatory and can relieve pain. Flavonoids can inhibit cyclooxygenase or lipoxygenase and inhibit the accumulation of leukocytes in the area of inflammation so that inflammation cannot occur. The results of research [8], ethanol extract of the bark of pulai can be used as an antioxidant. These antioxidants have pharmacological effects, one of which is anti-inflammatory [9, 10]. The antioxidant effect is one of the activities or effects of chemical compounds, namely flavonoids, where flavonoids have the ability to ward off free radicals in the body, as well as repair damaged body cells.

4 Conclusions and Suggestions

4.1 Conclusions

Based on the results of research on the activity test of the ethanol extract of the bark of pulai (Alstonia scholaris L.) as an anti-inflammatory in mice (Mus musculus), it was concluded that:

- Ethanol extract of pulai bark (Alstonia scholaris L.) has activity as an anti-pain in inflammation of mice (Mus musculus)
- The effective dose of the ethanolic extract of the bark of pulai (Alstonia scholaris L.) as an anti-pain in inflammation in mice (Mus musculus) is a dose of 200 mg/kgBW mice.

4.2 Suggestions

- Research on the anti-inflammatory activity test (pain symptoms) of the ethanolic extract of the bark of pulai (Alstonia scholaris L.) in mice test animals needs to be developed.
- To maximize the anti-inflammatory effect (pain symptoms) the ethanolic extract of the bark of pulai (Alstonia scholaris L.) is required another dose.
- For further researchers to pay more attention to the biological variation factor of the test animals because the possibility of influencing the data obtained is very large.

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