



ANTIPIRETIC ACTIVITY OF ETHANOL FRACTION OF PANDAN LAUT LEAVES (*Pandanus odorifer*) AGAINST MALE MICE (*Mus musculus*) INDUCED BY DPT-HB VACCINE

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ABSTRACT

Fever is a condition where the body temperature exceeds 37 °C caused by disease or inflammation resulting from a viral or bacterial infection. People consume synthetic drugs or commonly called antipyretics, to treat fever. This study aims to determine the antipyretic activity of the Ethanol fraction of sea Pandanus leaves (*Pandanus odorifer*) against male mice (*Mus musculus*) induced by the DPT-HB vaccine. Sample extraction was done by the maceration method with various solvent polarities (non-polar; n-Hexane, semi-polar; Ethyl Acetate, polar; Ethanol). All three fractions were tested for their secondary metabolites of Flavonoids with antipyretic activity. In addition, an antipyretic activity test was carried out on male mice induced by the DPT-HB vaccine-induced by an intramuscular method in the lower abdomen. An infrared thermometer (ANENG AN201) measured the mice's body temperatures every 30 minutes for 180 minutes after induction. Phytochemical identification results show that the n-Hexane fraction contains Tannins and Steroids as secondary metabolites. The Ethyl Acetate fraction contains Tannins, Triterpenes, Steroids, and the Ethanol fraction contains Tannins, Triterpenes, Steroids, Saponins, and Flavonoids. The antipyretic activity test results show that the Ethanol fraction of sea Pandanus leaves (*Pandanus odorifer*) has the potential to be antipyretic against mice (*Mus musculus*), and the most effective antipyretic effect was found in infraction with a dose of 600 mg/kg body weight.

Keywords: *Antipyretic, pandanus odorifer, Mice (Mus musculus), DPT-HB vaccine*

INTRODUCTION

Fever is a sign or symptom of a disease in the body. Some diseases, such as dengue fever, malaria, sore throat, and hepatitis, are diseases that start with a fever. Fever's adverse effects include dehydration, hypoxia, nerve damage, headaches, other discomforts, loss of appetite (anorexia), weakness and muscle aches. In order to

reduce this negative effect, fever needs to be treated with antipyretic drugs [1,2,3].

People usually take synthetic drugs from pharmacies to treat fever. Synthetic drugs, commonly referred to as antipyretics that are often used, are paracetamol (biogesic, bodrex junior, panadol, tempr) and types of aspirin (bodrexin, aspirin, bayer). The antipyretic most widely used to

treat fever and mild pain is paracetamol [4,5,6]. In addition to using synthetic drugs, fever treatment can also be done traditionally by using drugs derived from plants. Besides being cheap, traditional medicines have fewer side effects than synthetic drugs [3,7,8,9,10]—one of the plants with the potential as an antipyretic is the Pandanus plant. Research on *Pandanus tectorius* and *Pandanus amaryllifolius* has been reported many times. *Pandanus amaryllifolius* has reported its pharmacological activity as antibacterial, anti-diabetic, anticancer, and antioxidant. Solvents were varied for the extraction step, and activity was highest shown by ethyl acetate extract [8]. Reports on *Pandanus odorifer*, on the other hand, are limited to cytotoxic and antibacterial activity.

Pandanus is a plant. Various parts of its body have various benefits in daily life, including food ingredients, spices, dyes, weaving materials, roofs, cushions, medicines, and ornamental plants [11,12,13,14,15]. According to research by [8,9,10], *Pandanus tectorius* also has benefits in the health sector, namely as a toothache medicine, anti-inflammatory, antioxidant, anticancer, anti-tumour, anti-viral, anti-diabetic, and cholesterol-lowering activities. However, there are few reports on Pandan Leaves of *Pandanus odorifer* species, especially on their chemical content and activity. The leaves of *Pandanus odorifer* contain several secondary metabolites, namely Terpenoid, Flavonoid, Tannin, and Saponin. Leaf extract has a strong cytotoxic activity with an LC50 value of 4.3557 ppm using the BSLT method [16]. Pandan Laut (*Pandanus odorifer*) is a type of pandan plant that is widely found on the

Bengkulu coast. Sea pandanus tree habitats are on semi-natural beaches throughout the tropical and subtropical Pacific and can withstand drought and strong winds. The distribution of sea pandanus is found worldwide, including in Africa, Madagascar, India, and Indochina. The secondary metabolite profile of *Pandanus odorifer* ethanol extract has the potential as antipyretic and anti-inflammatory while its antipyretic activity has not been reported. Flavonoids have various anti-viral, anticancer, anti-inflammatory, antioxidant, anti-hepatotoxic, and anti-diabetic effects [8,18].

METHODS

1. Sample Preparation

Samples of *Pandanus* leaves were collected from the Sungai Suci Coast in the central regency of Bengkulu. The leaves are cleaned, chopped into small pieces, then air-dried, and then mashed with a blender until they become powder.

2. Extraction

Sample extraction was carried out by graded maceration with three solvents: n-Hexane, Ethyl Acetate, and Ethanol. This multilevel maceration uses solvents with different levels of polarity so that the extracted metabolites will be grouped according to the level of the polarity of the solvent. The sample in powder form was weighed as much as 1500 g and then soaked in n-hexane as solvent. Maceration with n-hexane was carried out several times, then the macerate was filtered and evaporated so that a thick fraction of n-hexane was obtained. This maceration work step was

repeated for the next solvent, ethyl acetate and Ethanol. Then the phytochemical profile test was carried out on the three fractions obtained.

3. Antipyretic Activity Test

The antipyretic activity test used a modified method that was carried out by [19]. This research begins with the preparation stage by adapting the test animals for one week and maintaining their condition. Next, make a 1% Na-CMC solution by weighing 1 g of Na-CMC, then put it little by little into a container that has been filled with 50 ml of hot distilled water (70OC) while stirring until a colloidal solution is formed and the volume is made up to 100 ml. The next step is to determine the dose and make a paracetamol solution. The dose of paracetamol used was 65 mg/kg BW, which was obtained from the following calculations:

$$\frac{500 \text{ mg} \times 0,0026}{20 \text{ g}} = 1,3 \text{ mg}/20\text{gBB mice}$$

$$\frac{1,3 \text{ mg}}{20 \text{ g}} \times 1000 = 65 \text{ mg}/\text{kgBB mice}$$

The paracetamol solution was carried out 65 mg of paracetamol powder and then put into a beaker, adding a little 1% Na-CMC solution, and stirred until homogeneous. Then put the homogeneous solution into a 10 mL volumetric flask and make up the volume to the mark with 1% Na-CMC solution. The volume of the solution given orally to mice was:

$$\frac{20}{1000} \times 10 \text{ mL} = 0.2 \text{ mL}/\text{kgBB mice}$$

Furthermore, making the fraction solution, the fraction was divided into three dose groups, namely doses of 200; 400; 600 mg/kg BW. The solution was made utilizing ethanol extract of pandan Laut leaves weighed 200,

400, and 600 mg, then dissolved using a 1% Na-CMC solution. The volume was made up of 1% Na-CMC solution in a 10 mL volumetric flask, and what would be given orally was the same as that given by mouth paracetamol.

Antipyretic activity testing was done by dividing the test animals into five groups: the P0 group: P1, P2, P3, and P4. The mice will be fasted first from food but still given water for 8 hours. Then the mice are weighed, and their temperature is measured. After that, the mice were induced with the DPT-HB vaccine and allowed to stand for 30 minutes. After 30 minutes, the temperature of the mice will be measured again if it is confirmed that the fever in the mice will be given a fraction. According to the test group, namely:

P0: negative control

P1: Positive control

P2: dose fraction 200 mg/kg BW

P3: dose fraction 400 mg/kg BW

P4: dose fraction 600 mg/kg BW

After The mice were treated according to the test group, the temperature of the mice was measured every 30 minutes for 180 minutes.

RESULTS AND DISCUSSION

Identification of secondary metabolites of Alkaloids was not detected (-) in each of the pandan sea leaf fractions in the phytochemical test because no white precipitate was found. In the alkaloid test, HCl was added before adding the reagent because the Alkaloid was alkaline, so it was extracted with a solvent containing acid [20]. The formation of a white precipitate indicates a positive result in the Meyer test. This reaction occurs when using Mayer's reagent

so that a white precipitate is formed, K⁺ metal ions will form covalent bonds with Nitrogen atoms so that they can form complex compounds [21]. The results of the tannin test were detected (+) in each fraction in the phytochemical test because the color changed to blackish green. Tannins are compounds with several phenolic hydroxy groups that are widely found in plants [22].

The Saponin test was only detected (+) in the Ethanol fraction, while the n-hexane and ethyl acetate fractions were not detected (-). Detection of this compound is indicated by the presence of permanent foam and does not disappear. Saponins are polar, so they can dissolve in solvents such as water. However, it is non-polar because it has a hydrophobic group, namely an aglycone (sapogenin). This secondary metabolite has several biological activities, namely lowering blood cholesterol levels [23,24].

Triterpenoid test using the Liebermann-Burchard method showed positive results indicated by the presence of a brownish or violet ring at the boundary of the added solvent. In this study, positive results were obtained in the presence of a brownish ring. Terpenoid derivative compounds have anti-microbial and anti-fungal activity [25]. Sea pandan leaf Steroids, as well as Triterpenoid compounds, these compounds were only detected (+) in the Ethyl Acetate and Ethanol fractions. Steroids are Triterpenoid compounds. A change in the extract indicates a positive test for this compound to blue or green. Steroid tests on Pandan Laut leaves are characterized by a change in color to green. Steroid tests on sea pandan leaves are characterized by a change in color to green.

The green colour on the Steroid test (i.e. cholesterol) was caused by a reaction between the hydroxyl functional group on the C3 position with the Liebermann Burchard reagent, resulting in 3-aceto-5-cholesterol sulfonate acid [26]. The Flavonoid test uses the Bate Smith & Metcalf method. The function of concentrated Mg and HCl metals in this test serves to reduce the Benzopyron core contained in the Flavonoid structure so that a color change becomes dark red or orange. The results of the phytochemical screening test can be seen in Table 1.

Antipyretic activity in this study used male mice (*mus musculus*) swiss webster strain aged 8-12 weeks obtained from the SBIH Ruyani laboratory in Bengkulu. White male mice used were mice with a weight of 22-27 g that had been adapted for seven days and fasted from food for 8 hours before testing. Furthermore, to determine whether or not there is an antipyretic effect of ethanol extract from sea pandan leaves, the study was conducted on mice with fever.

Mice were given the DPT-HB vaccine intramuscularly to increase temperature and to determine whether the mice had a fever or not; the temperature of the mice was measured before and after the DPT-HB vaccine was induced. They measured the temperature of mice using an infrared thermometer (ANENG AN201). Changes in the temperature of the mice can be seen in Table 2.

Table 2 data shows that the temperature of the mice increased after 30 minutes of being induced by the DPT-HB vaccine; this indicates that the DPT-HB vaccine has worked well on the mice, causing

the mice to become feverish. Furthermore, the temperature of the mice after being treated with each test group can be seen in Table 3 and Figure 1.

Table 1. Phytochemical screening results

| Chemical content | Method | Positive test | Hexan Faction | Fraction Ethyl acetate | Fraction Ethanol |
|------------------|---|-----------------------|---------------|------------------------|------------------|
| Alkaloids | Mayer | White precipitate | - | - | - |
| Tannins | FeCl ₃ | Blackish green | + | + | + |
| Saponins | Forth. test | foaming | - | - | + |
| Triterpenoids | Chloroform + acetic anhydrous | brown ring | - | + | + |
| Steroids | H ₂ SO ₄ concentrated | Green or Blue | + | + | + |
| Flavonoids | Bate Smith & Mertcalf | Red, yellow or orange | - | - | + |

Table 2. Average temperature increase of mice after DPT-HB vaccine was induced.

| Group | Before vaccine induction (0C) | 30 minutes after vaccine induction (0C) | Average temperature rise (0C) |
|----------------|-------------------------------|---|-------------------------------|
| Na-CMC | 36.4 | 37.5 | 1.1 |
| Paracetamol | 36.7 | 37.7 | 1.0 |
| 200 mg/kg BW | 36.6 | 37.6 | 1.0 |
| 400 mg/kg BW | 36.7 | 37.7 | 1.0 |
| 600 mg/kg BW | 36.7 | 37.5 | 0.8 |
| Average amount | | | 0.98 |

Table 3. The average temperature of mice every 30 minutes after being given treatment

| Group | minute- | | | | | | |
|--------------|--|------|------|------|------|------|------|
| | 0 | 30 | 60 | 90 | 120 | 150 | 180 |
| | The average temperature of each group of mice (0C) | | | | | | |
| Na-CMC | 37.5 | 37.4 | 37.9 | 38.0 | 38.5 | 38.3 | 38.6 |
| Paracetamol | 37.7 | 37.3 | 37.3 | 37.2 | 37.5 | 37.3 | 37.2 |
| 200 mg/kg BW | 37.6 | 37.3 | 37.4 | 37.2 | 37.4 | 37.2 | 37.3 |
| 400 mg/kg BW | 37.7 | 37.4 | 37.3 | 37.3 | 37.5 | 37.2 | 37.2 |
| 600 mg/kg BW | 37.5 | 37.1 | 37.0 | 37.0 | 37.2 | 37.2 | 37.0 |

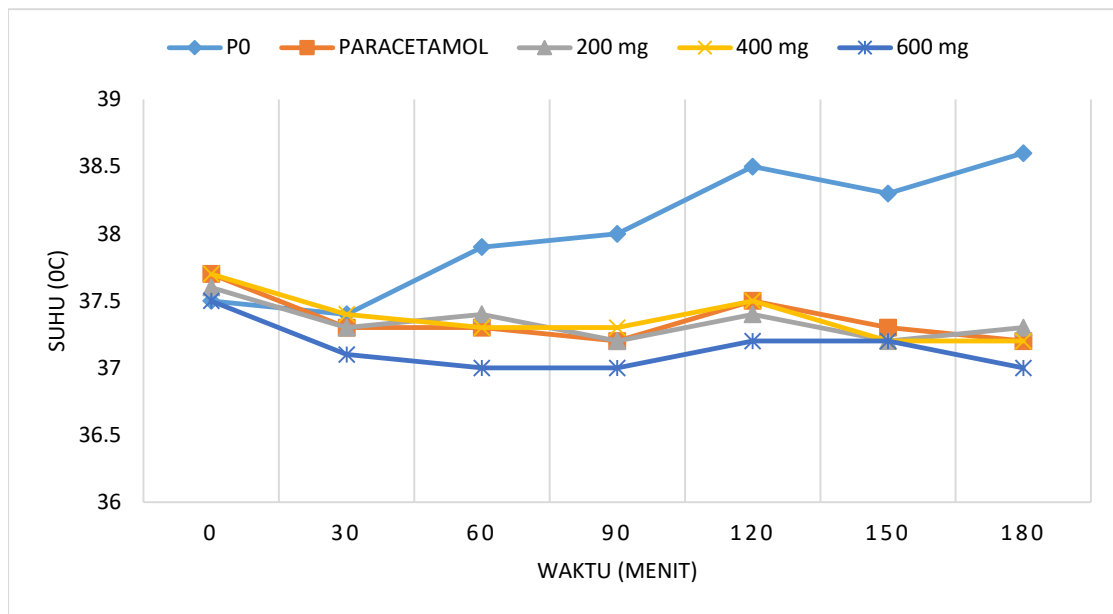


Figure 1. Graph of the average temperature measurement of mice every 30 minutes (°C) after being given treatment

Description: *minute 0: vaccine induction

*30th minute: administration of test preparations

Based on Table 3, the average temperature of the mice every 30 minutes after being treated for each group. This graph is useful to provide a clearer picture of temperature changes that occur every 30 minutes in each treatment group. The graph of the average temperature of mice every 30 minutes after being given treatment for each group is in Figure 1. Based on Figure 1, the greatest decrease in temperature was found in the paracetamol group at a dose of 400 and a dose of 600 mg/kg BW. the group given paracetamol experienced a large decrease in temperature, this decrease in temperature was because paracetamol is a drug that has an antipyretic effect. The average decrease in temperature of mice in each experimental group is shown in Table 4.

Table 4 shows that the fractions of 400 mg/kg BW and 600 mg/kg BW have the same decrease in temperature of 0.5 °C. When

viewed from the process of decreasing temperature in the dose group of 600 mg/kg BW, there was a regular decrease until the 180th minute. The 400 mg/kg BW dose group experienced an irregular decrease in the temperature of the mice and a decrease in the temperature of the mice that stopped at 1 minute. Based on this, it can be concluded that the dose group of 600 mg/kg BW had a more optimal antipyretic effect than the dose group of 200 mg/kg BW and 400 mg/kg BW. The dose of 600 mg/kg BW of the ethanol fraction of sea pandan leaves had a stronger antipyretic effect in vaccine-induced mice. The antipyretic effect of the ethanol fraction of sea pandan leaves is thought to be due to the presence of flavonoid compounds contained in sea pandan leaves. Several types of compounds in flavonoids have various bioactivities that have antipyretic effects [7]. Antipyretic is the activity of

chemical compounds in reducing fever by inhibiting the cyclooxygenase enzyme that catalyzes the formation of prostaglandins, compounds that cause fever. Metabolic compounds such as flavonoids in plant extracts can inhibit lipoxygenase and COX enzymes. This compound also inhibits the biosynthesis of prostaglandins and leukotrienes, both of which are active ingredients in increasing fever.

Data on the average temperature decrease in Table 4, statistical tests were carried out to determine the effect of dose variations of the ethanolic extract of pandan Laut leaves on the average decrease in body temperature of test animals induced by the DPT-HB vaccine. Normality test obtained a significance value of 0.574 (Na-CMC); 0.067 (paracetamol); 0.307 (200 mg/kg BW); 0,292 (400 mg/kg BW) and 0,055 (600 mg/kg BW).

Significance value > 0.05, which means the data is normally distributed. Furthermore, the homogeneity test obtained a significance value of 0.006 <0.05, indicating that the data is not homogeneous. Because the results of the prerequisite test did not meet the requirements for homogeneous data, the analysis was carried out using a non-parametric test using the Kruskal Wallis test, as shown in Table 5.

Significance value or Asymp value. Sig. The results obtained in table 5 are 0.001 <0.05, so it can be concluded that the dose variation of the ethanolic extract of pandan Laut leaves decreases the temperature of mice induced by the DPT-HB vaccine. The following statistical test was the Mann-Whitney test which was conducted to determine whether there was a significant difference in the average decrease in body

Table 4 The average decrease in temperature of mice (0C) from minute 0 to minute 180

| Group | Average temperature drop (0C) | | | | | | Total average temperature (0C) |
|--------------|-------------------------------|-------|-------|--------|---------|---------|--------------------------------|
| | 0-30 | 30-60 | 60-90 | 90-120 | 120-150 | 150-180 | |
| Na-CMC | 0.1 | -0.5 | -0.1 | -0.5 | 0.2 | -0.3 | -1.1 |
| Paracetamol | 0.4 | 0.0 | 0.1 | -0.3 | 0.2 | 0.1 | 0.5 |
| 200 mg/kg BW | 0.3 | -0.1 | 0.2 | -0.2 | 0.2 | -0.1 | 0.3 |
| 400 mg/kg BW | 0.3 | 0.1 | 0.0 | -0.2 | 0.3 | 0.0 | 0.5 |
| 600 mg/kg BW | 0.4 | 0.1 | 0.0 | -0.2 | 0.0 | 0.2 | 0.5 |

Table 5 Summary of test results Kruskal walis

| Fever temperature | |
|-------------------|--------|
| Chi-Square | 17.925 |
| df | 4 |
| asypm. Sig. | .001 |

Table 6 Asimp Sig Value Mann-Whitney test results for each group comparison

| Nilai Asimp. Sig | Na-CMC | Paracetamol | 200 mg/kg BW | 400 mg/kg BW |
|------------------|--------|-------------|--------------|--------------|
| Paracetamol | .007 | | | |
| 200 mg/kg BW | .006 | .947 | | |
| 400 mg/kg BW | .009 | .842 | .844 | |
| 600 mg/kg BW | .003 | .026 | .032 | .027 |

Temperature of the test animals induced by the DPT-HB vaccine. The Mann-Whitney test was carried out by comparing each group's data one by one if the Mann-Whitney test results obtained asymp value. Sig. <0.05 means a significant difference, while the asymp value is. Sig. >0.05 means that there is no significant difference between the test groups. The results of the Mann-Whitney test on groups of test animals were carried out with asymp value output. Sig obtained can be seen in Table 6.

Results of the Mann-Whitney test as shown in table 6, several groups of test animals had asymp values. Sig. <0.05, which means there is a significant difference in the average decrease in body temperature of test animals induced by the DPT-HB vaccine between groups of test animals. The most significant difference was found in the 600 mg/kg BW group and the Na-CMC group with asymp values. Sig. 0.003.

Based on the results of the two data analyses, namely the Kruskal Wallis test and the Mann-Whitney test, it can be stated that the ethanol fraction of sea pandan leaves (*Padanus driver*) has potential as an antipyretic. This antipyretic activity is thought to come from secondary metabolites found in the ethanol fraction, one of which is flavonoids, where according to [18], have several roles such as antiinflammation/ antipyretic by inhibition of cyclooxygenase-2

(COX2); antibacterial by inactivation of microbe's adhesins, enzymes, and proteins; anticancer by apoptosis induction that stop cell cycles; and antioxidant by reducing free radicals [28].

CONCLUSION

The results of the phytochemical test of the ethanol fraction of Pandan Laut (*Pandanus odorifer*) leaves on the positive n-hexane fraction containing tannins and steroids. The positive ethyl acetate fraction contains tannins, steroids and triterpenoids. The positive ethanol fraction containing tannins, steroids, flavonoids, saponins, triterpenoids and based. The results of the antipyretic test and statistical tests carried out, it can be concluded that the ethanol fraction of sea pandan leaves at a dose of 200; 400; 600 mg/kg BW in general has antipyretic activity with the most optimal antipyretic activity at a concentration or dose of 600 mg/kg BW.

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