

# Content of Banten Leaf Extract (*Lannea Coromandelica* (Houtt.) Merr.) Traditional Medicine of Lombok as an Antipyretic in Mice (*Mus Musculus*)

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**Abstract:** The Sasak tribe, who are the indigenous inhabitants of Lombok Island, use banten (*Lannea coromandelica* (Houtt.) Merr.) leaves as a medicinal plant to reduce fever. Fever is a physiological response to infection, characterized by a body temperature above the normal range of approximately  $\pm 37$  °C. If left untreated, fever can cause dehydration, lack of oxygen, nerve damage, dizziness, decreased appetite, weakness, and muscle pain. To minimize these effects, antipyretics such as paracetamol can be used. However, prolonged or excessive use of paracetamol can cause hepatotoxicity, hepatic necrosis, renal tubular necrosis, and hypoglycemic coma. Therefore, it is necessary to develop alternative herbal medicines to treat fever. The aim of this study is to determine the content and antipyretic effect of banten leaf extract, which was obtained through ethanol extraction. The antipyretic ability test utilized five groups: the negative control group, positive control group, and test groups receiving doses of 1 mg/25gBB, 3 mg/25gBB, and 10 mg/25gBB of ethanol extract of banten leaves. Fever induction was induced using the DPT-HB vaccine. The study found that the ethanol extract of banten leaves contained flavonoids (572.38 mg QE/gram extract), phenols (200.82 mg GAE/gram extract), and antioxidants (2.5063 mg GAE/gram extract with DPPH). The percentage of antioxidant inhibition in banten leaves between 9.68-47.16 %. Additionally, all test groups of the ethanol extract of banten leaves exhibited antipyretic activity. The antipyretic effect of the 3 mg/25gBB dose was equivalent to that of paracetamol. Similarly, the antipyretic effect of the 10 mg/25gBB dose was almost identical to that of paracetamol at 5 hours after treatment.

**Keywords:** Antipyretic; Banten leaves (*L. coromandelica*); DPT-HB vaccine; Fever; Traditional medicine

## Introduction

Indonesia is a country with high biodiversity, particularly in flora. According to Lestari et al. (2018), 26% of plants in Indonesia have been cultivated while the rest grow wild in various Indonesian forests. It is

noteworthy that more than 9,609 species of Indonesian plants have medicinal properties, with 940 of them being widely used as medicines. The use of plants as medicinal materials is one of the many benefits of plants (Yassir & Asnah, 2019).

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Medicinal plants have been used for centuries to treat diseases and have played a significant role in human civilization (Sultana et al., 2015). Prior to the development of synthetic drugs, humans relied on the medicinal properties of various parts of plants (Essien et al., 2015). One such plant is the banten plant (*L. coromandelica* (Houtt.) Merr.), which has natural medicinal properties. The tropical deciduous tree, *Lannea*, belongs to the Anacardiaceae family and is widely distributed in India, Bangladesh, and other tropical countries (Alam et al., 2017). Traditional medicine practitioners in rural Bangladesh prescribe the leaves and bark of this plant to patients suffering from diarrhea and other diseases (Hossain et al., 2018). Furthermore, the plant in Bangladesh is utilized for treating swelling, localized pain, inflammation, skin diseases, and cancer (Rahman et al., 2016).

The Sasak tribe, an indigenous group inhabiting Lombok Island, employs banten leaves as a medicinal plant to reduce fever. Fever is a condition in which the body temperature rises above normal approximately  $\pm 37^\circ\text{C}$  in response to an infection (Rezaldi et al., 2022). If left untreated, fever can lead to dehydration, oxygen deprivation, nerve damage, dizziness, decreased appetite, weakness, and muscle pain. To minimize these effects, antipyretics such as paracetamol can be used to reduce fever. However, prolonged or excessive use of paracetamol can cause hepatotoxicity, hepatic necrosis, renal tubular necrosis, and hypoglycemic coma (Desiana et al., 2018). To minimize or eliminate these side effects, consider exploring natural alternatives such as traditional medicine (Azis, 2019b).

Banten plant, typically used as a residential fence plant, has been analyzed in previous studies. The analysis revealed the presence of various compounds, including phenolic compounds, glycosides, quinones, terpenoids, alkaloids, saponins, flavonoids, triterpenoids, steroids, and tannins (Selvaraj et al., 2015). The ethanol extract of Banten leaves was reported to have hepatoprotective potential and antioxidant activity. The extract contains phenolic compounds, terpenoids, and alkaloids, which are bioactive compounds that exhibit antioxidant, anti-inflammatory, antibacterial, anticancer, and immunomodulatory activities (Rao et al., 2014; Nasrudin et al., 2021).

Research by Rezaldi et al. (2022) testing antipyretic activity on *kaca piring* leaves showed that flavonoids can reduce body temperature by inhibiting the enzyme cyclooxygenase-2 (COX-2) in prostaglandin biosynthesis so that fever can be inhibited. Flavones and flavonols as a class of flavonoids have antipyretic activity because they have a benzene group similar to paracetamol. The antipyretic ability possessed by paracetamol is caused by the aminobenzene group. Based on the experience of the community, this study aims to determine the content

and antipyretic ability of ethanol extract of banten leaves (*L. coromandelica* (Houtt.) Merr.) Lombok traditional medicine needs to test the activity of its compounds in male mice (*Mus musculus*). This is done as a scientific test of the ability of these traditional medicines to reduce fever.

## Method

### Sample Preparation

Samples of wild bantam leaves were taken and then stored in a ziplock. Next, the sample was photographed using an Olympus Waterproof camera. The morphology of Banten leaves was identified based on The World Flora Online and literature studies. Banten leaves have the morphological characteristics of odd-pinnate compound, green in color, smooth leaf surface, flat leaf edges, opposite leaflets, pinnate leaf veins, oval leaf structure (ovatus), pointed leaf tip and blunt leaf basal (obtusus). Suitable banten leaf samples are stored in the herbarium of the Center for Bioscience and Biotechnology (PUBB), Mataram University, each as a specimen with vouchers PUBBH\_TP\_01, PUBBH\_TP\_02, and PUBBH\_TP\_03. The collected bantam leaves are washed with tap water to remove unwanted dirt and sand. Making *Simplisia A* total of 1,000 g of Banten Banten leaves were dried using a dryer at a temperature of  $35^\circ\text{C}$  for 24 hours and occasionally turned over. Once dry, cut and blend until it becomes powder.

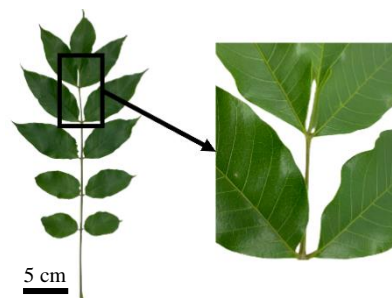


Figure 1. Banten leaf morphology

### Making *Simplisia*

A total of 1,000 g of Banten palm leaves were dried using a dryer at  $35^\circ\text{C}$  for 24 hours and occasionally turned over. Once dry, cut and blend until it becomes powder.

### Making Banten Leaf Extract

200 g of banten leaf powder was filled into a beaker and then sonicated with 1 liter of 96% ethanol for 80 minutes. After that, the same procedure was carried out twice so that the total ethanol used was 3 L.

## Test the Content of Banten Leaf Extract Using UV-VIS Spectrophotometry

### Measurement of Total Phenols

Measurement of the total phenols of banten leaf extract used gallic acid solution (GAE) as a standard and was made by dissolving 10 mg in 10 mL of ethanol (1 mg/mL). Various concentrations of GAE (10–500 µg/mL) were prepared from stock solutions. Approximately 100 µL of sample (1 mg/mL) was combined and mixed with 0.75 mL of Folin-Ciocalteu reagent. The solution mixture was incubated for 5 minutes at room temperature. To this solution, 750 µL of sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) was added. After 90 minutes, the absorbance of the solution was measured at a wavelength of 725 nm using a UV-Vis spectrophotometer (Multiskan-Go, Thermo Scientific) (Prasedya et al., 2021).

### Measurement of Total Flavonoids

Total flavonoid content using Quercetin as a standard solution. Approximately 100 µL of sample was added to 4 mL of dH<sub>2</sub>O. Then followed by the addition of 300 µL of 5% sodium nitrite. After 5 min, 300 µL of 10% aluminum chloride was added. The mixture was incubated for 6 minutes before adding 2 mL of 1 M sodium hydroxide. Immediately, the mixture was diluted by adding 3.3 mL of dH<sub>2</sub>O and stirring. The absorbance of the sample was measured with a wavelength of 510 nm (Prasedya et al., 2021).

### DPPH Antioxidant Activity Test

The DPPH (2,2-diphenyl-1-picrylhydrazyl) test uses gallic acid as a standard solution to create a calibration curve. Samples with a concentration of 0.1% were dissolved in absolute alcohol. A volume of 100µL of extract was mixed with 100µL of DPPH reagent. Absorbance was measured at a wavelength of 517 nm using a spectrophotometer (Prasedya et al., 2023).

### In-Vivo Test Design

DPT-HB vaccine, DPT-HB vaccine injected 0.03 ml/25gBW of mice, Negative Control (distilled water) 0.3 ml of distilled water was given, Positive Control (Paracetamol Syrup), Paracetamol syrup given as much as 0.3 ml/25gBW of mice, Banten Leaf Extract Treatment Dose 1 was given banten leaf extract as much as 1 mg/25gBW of mice. Then dose 2 is 3 mg/25gBW of mice. And dose 3 is 10 mg/25gBW of mice.

### In-Vivo Antipyretic Testing

All male mice had their body temperature measured using a rectal thermometer, right before being injected with the DPT-HB vaccine which will induce fever. Male mice were injected with DPT HB vaccine 0.03 mL/25 gr BW intramuscularly in the thigh. Two hours

after being given the vaccine, their body temperature was measured using a rectal thermometer and after the mice had a fever or their body temperature exceeded normal limits, each group was treated orally using a 0.3 mL solution, namely: Group A: Negative control using distilled water. Group B: Positive control using paracetamol syrup. Group C: Treatment dose of 1 banten leaf extract is 1 mg/25gBW for mice. Group D: Treatment with a dose of 2 banten leaf extracts of 3 mg/25gBW for mice. Group E: Treatment dose of 3 banten leaf extracts of 10 mg/25gBW for mice. After being given treatment, 1 hour later the rectal temperature was measured again until the experimental time reached 5 hours with 2 hour intervals.

### Statistic Analysis

Data obtained from observations are presented in table form by including the average of each treatment and repetition carried out. Next, the data was subjected to a One-Way Anova test to determine the effect of banten leaf extract on reducing the body temperature of male mice induced by the DPT-HB vaccine with a confidence level of 95%. Further tests were carried out with LSD to determine the real differences in each treatment

## Result and Discussion

Banten leaves were macerated using 3000 mL of 96% ethanol. The 96% ethanol solvent is used because it is selective, neutral, non-toxic, well absorbed, can prevent the growth of mold and bacteria, is easy to evaporate, and the required temperature is lower so as to minimize the risk of compound shrinkage due to heating (Suharyanto & Hayati, 2021). The weight of the ethanol extract obtained from banten leaves was 4.98 grams with a soaking of 2.50% in 200 grams of dry powder. Soakment analysis is carried out to determine the levels of secondary metabolites carried by the solvent, but cannot determine the type of compound.

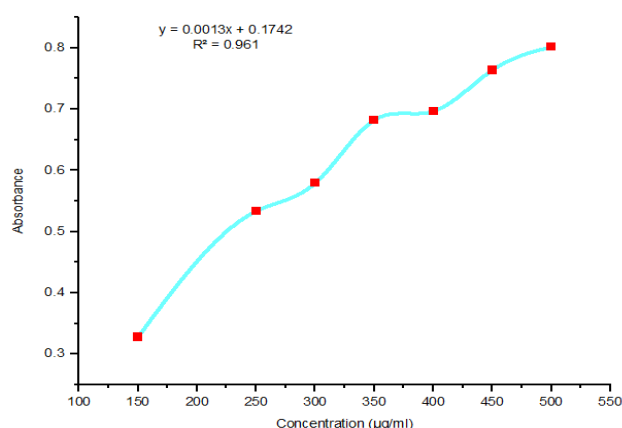


Figure 2. Quercetin standard curve

This research carried out quantitative tests to determine the content of flavonoids, phenols and antioxidants (DPPH) in Banten leaf extract using UV-vis spectrophotometry. The results of measuring the total absorbance of flavonoids for the Quercetin calibration curve showed that the regression equation was  $y = 0.00x + 0.17$ . Quercetin as a standard flavonoid solution obtained a correlation coefficient value of  $R^2=0.96$ . The value of  $r = 0.9803$  is close to one, indicating that the regression equation and graph formed have a correlation between absorbance and concentration according to Figure 2. The curve equation was used to determine the total flavonoid content in Banten leaf extract with a result of 572.38 mg QE/gram of extract, meaning that in for every 1 gram of ethanol extract of Banten leaves, there are flavonoids equivalent to 572.38 mg of Quercetin.

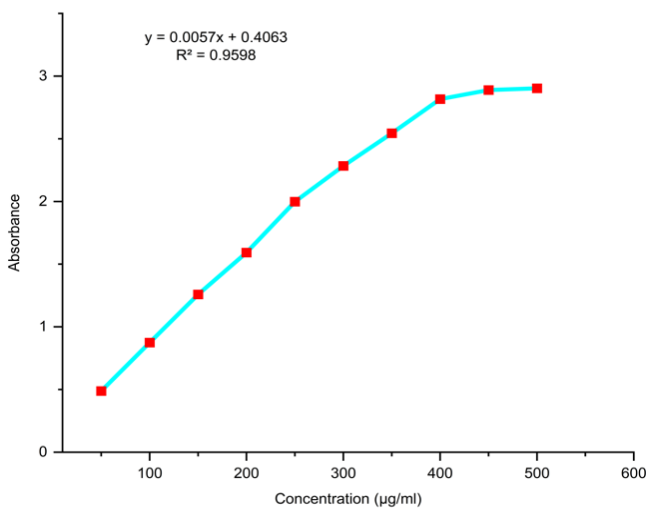


Figure 3. Gallic acid (phenol) standard curve

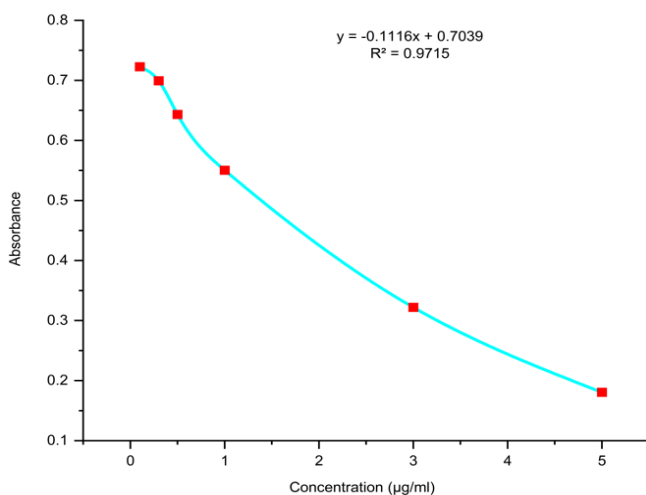


Figure 4. Standard curve of gallic acid (DPPH)

The results of measuring the absorbance of a standard solution of gallic acid to create a total phenol calibration curve obtained a regression equation of  $y=0.0057x+0.4063$  and a correlation coefficient value of  $R^2 = 0.9598$ . The value of  $r = 0.9797$  is close to one, indicating that there is a correlation between absorbance and concentration according to the equation and graph formed in Figure 3. Based on this equation, the total phenolic content of Banten leaf extract is 200.82 mg GAE/gram of extract, meaning that in every 1 gram of Banten leaf ethanol extract there is phenolic compounds equivalent to 200.82 mg gallic acid.

The results of measuring the absorbance of a standard solution of gallic acid to create a calibration curve for antioxidant activity obtained the regression equation  $y = -0.11x+0.70$ . Gallic acid as a standard solution for antioxidant activity was obtained with a correlation coefficient value of  $R^2 = 0.97$ . The value of  $r = 0.98$  is close to one, indicating that there is a correlation between absorbance and concentration according to the graph and equation formed in Figure 4. Based on this equation, the concentration of antioxidants contained is 2.5063 mg GAE/gram of extract, so that the percentage of antioxidant inhibition in Banten leaves is among 9.68 - 47.16%. This percentage contains antioxidants which are classified as low, said to be moderate if it reaches 50%, and considered high if it reaches 80% (Loganayaki et al., 2013).

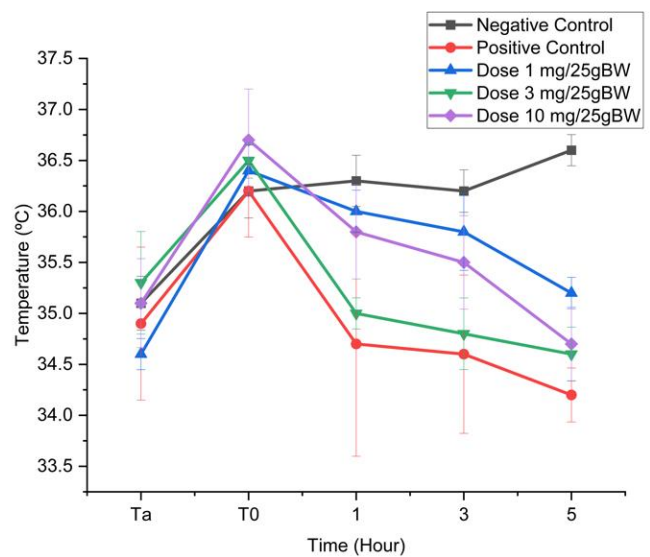


Figure 5. Temperature change graph

Measuring the average body temperature of mice before being induced by fever was found to be 35.1 °C. After that, it is induced using the DPT-HB vaccine. The DPT-HB vaccine contains the Bordetella pertussis microbe, causing the body's defense response, namely mononuclear cells releasing pro-inflammatory cytokines to influence the hypothalamic thermoregulation center

to increase body temperature (Jansen et al., 2015). This can be seen by the average increase in body temperature of mice after injection of 36.4 °C. Each test group was measured for a decrease in body temperature at 1 hour, 3 hours and 5 hours after being treated. The results are based on the graph (Figure 5.) At 1-5 hours all test groups except the negative control experienced a decrease in temperature. The data was analyzed using statistical tests of normality, homogeneity and LSD.

The data obtained were normally distributed ( $p > 0.05$ ) and the variations were homogeneous ( $p > 0.05$ ). The LSD test showed that there was a significant difference in each test group compared to the negative control ( $p < 0.05$ ). This proves that all groups have antipyretic activity. Positive control data for other treatments showed significant differences ( $p < 0.05$ ) except for the banten leaf extract group at a dose of 3 mg/25gBW which was not significantly different ( $p > 0.05$ ). So, the banten leaf extract group at a dose of 3 mg/25gBW had the same effect as paracetamol.

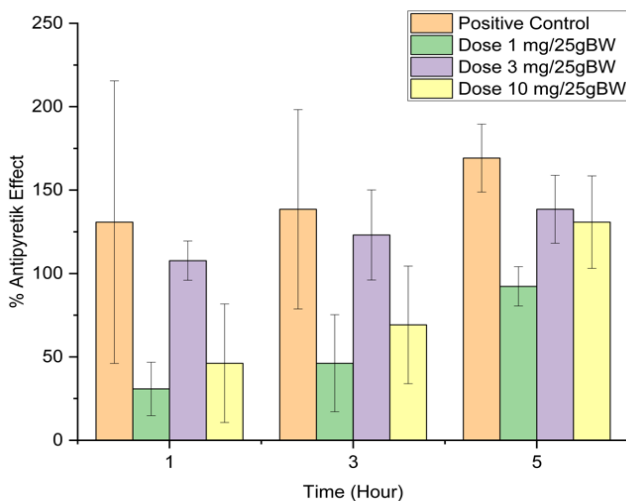


Figure 6. Antipyretic effect graph (%)

Antipyretic effect is a calculation carried out to determine the percentage of antipyretic effect caused by a substance. Based on the graph Figure 6 all test groups had an antipyretic effect. The positive control paracetamol produced the highest antipyretic power at each test time with 5 hours after treatment providing the highest results compared to other treatments. A dose of 3 mg/25gBW had the highest antipyretic effect among the treatment extract doses given, almost equaling the effect of paracetamol. So, a dose of 3 mg/25gBW is the most optimal dose in reducing fever in mice because its antipyretic ability is not much different from paracetamol. A dose of 10 mg/25gBW has almost the same antipyretic effect as a dose of 3 mg/25gBW at 5 hours after treatment. This is because the large dose given takes longer for the mice's body to process it, thus

providing a high antipyretic effect. A dose of 1 mg/25gBW had the smallest antipyretic effect at each test time due to the low dose of extract used for the mice's bodies so it did not provide a large antipyretic effect.

Increased body temperature occurs due to PGE2 binding to EP3 receptors in the median preoptic hypothalamus (Roy et al., 2019). The production of prostaglandins causes fever so that to provide an antipyretic effect is done by inhibiting prostaglandin synthesis through blocking the cyclooxygenase (COX) enzyme (Hassan et al., 2015). Substances that cause fever are called pyrogens, either originating from within the body (endogenous) or originating from outside the body (exogenous). In this study, fever was triggered exogenously because it used inactivated microorganisms to increase body temperature. These microorganisms are obtained from the DPT-HB vaccine. The DPT-HB vaccine is a toxoid vaccine derived from inactivated bacteria and viruses (Sambou, 2022).

Exogen's that enter the body will attach to immunoglobulins and are then phagocytosed by macrophages. This process releases pro-inflammatory cytokines such as IL-1, IL-6, TNF- $\alpha$ , Interferon (IFN) which act on the hypothalamus. Cytokines cause the release of arachidonic acid which is then converted into prostaglandins through the role of the enzyme cyclooxygenase 2 (COX-2). This prostaglandin is formed directly and can also be formed through the release of cyclic adenosine phosphate (cAMP) which then increases the thermostat temperature in the central nervous system and causes fever (Azis, 2019a).

Fever is treated using synthetic drugs as antipyretic agents including diclofenac, aspirin, and paracetamol (Mworia et al., 2019). However, synthetic drugs are still a problem so that antipyretic drug candidates with a good level of safety for consumption are needed (Jaffal et al., 2021). Through this study, banten leaves as a traditional Lombok medicine can reduce body temperature showing the presence of components that regulate the occurrence of fever by PGE2 through COX in the hypothalamic preoptic zone or fever by PGE2 through PPF, CRF, and ET-1 or both. Previous research shows that flavonoids can suppress TNF- $\alpha$  expression (Arya et al., 2019).

Based on the literature, the type of flavonoid bioactive compounds that have antipyretic effects with TNF- $\alpha$  suppression is baicalin (Abbasi et al., 2018). Flavonoids contained in banten leaf extract are the largest phenol compounds as antioxidants and also immunomodulators. Some studies show that flavonoids have good analgesic, anti-inflammatory, and antipyretic properties (Elshamy et al., 2019). The presence of flavonoids in banten leaf extract has the potential to influence antipyretics. The antipyretic mechanism is

possessed by secondary plant metabolites, namely flavonoids which have a compound structure similar to paracetamol. Flavonoids are able to inhibit the production of prostaglandins, causing an antipyretic effect (Saleem et al., 2015). Prostaglandins are inhibited by inhibiting the action of the cyclooxygenase enzyme that arises due to inflammation so that increased vasodilation in the skin occurs and heat from the body can be removed with increased sweating which causes body temperature to decrease (Widyaningrum & Ningrum, 2021).

Inhibition of the production of cyclooxygenase enzymes, especially COX-2, has an influence on eicosanoid hormones as biologically active substances from arachidonic acid such as thromboxane and prostaglandins. This mechanism lowers the body's thermostat point in the hypothalamus so that the fever goes down. Apart from the flavonoid content, the antioxidants activity in banten leaf extract also act as an antipyretic. Antioxidants work as inhibitors of prostaglandin biosynthesis in the microvascular endothelium in inhibiting the release of arachidonic acid and the secretion of lysosomal enzymes by blocking the COX pathway thereby reducing prostaglandin levels in the body (Widyasari et al., 2018).

## Conclusion

Based on the compound content of the ethanol extract of banten leaves using UV-vis spectrophotometry, the total flavonoid content was 572.38 mg QE/gram of extract, total phenol was 200.82 mg GAE/gram of extract, and antioxidants with DPPH were 2.5063 mg GAE/gram of extract, so that the percentage of antioxidant inhibition in banten leaves is between 9.68% - 47.16%. All test groups of banten leaf ethanol extract had antipyretic activity. A dose of 3 mg/25gBW shows an antipyretic effect that is similar to paracetamol. Then a dose of 10 mg/25gBW has an antipyretic effect that is almost the same as paracetamol 5 hours after treatment.

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## Author Contributions

Conceptualization; W. Q., N. H., Z. I., E. S. P.; methodology; W. Q.; validation; N. H.; formal analysis.; Z. I., investigation.; E. S. P resources; N. H; data curation: writing – original; W. Q, draft preparation; N. H., writing – review and editing; Z. I. Visualization; E. S. P. All authors have read and agreed to the published version of the manuscript.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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