

Research article

Anti-Inflammatory Activity of the Peel Extract of Ambon Bananas (*Musa Paradisiaca L.*) Examined With the Human Red Blood Cell Membrane Stabilization Method

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Abstract.

Inflammation is the body's reaction to the presence of infection, irritation or foreign substances as part of the body's defense mechanisms. Public interest in treatment with natural medicines is increasing. One of the medicinal plants that can be used for anti-inflammatory treatment is the peel of the Ambon banana (*Musa paradisiaca L.*). This study employed the red blood cell membrane stabilization method which is widely used in research as a biochemical parameter for in vitro testing of anti-inflammatory activity. This was an experimental study that aimed to determine the anti-inflammatory activity of the peel extract of the Ambon banana (*Musa paradisiaca L.*) using the red blood cell membrane stabilization method. The percentage level of hemolysis that occurred when the extract was added indicated that the tested extract had anti-inflammatory properties. The stability of the red blood cell membrane of the peel extract with a concentration of 125 ppm was 1.09%, and was 12.40% with a concentration of 250 ppm, 17.36% with a concentration of 500 ppm, and 42.79% with a concentration of 1000 ppm. Based on these results, it can be concluded that the peel of the Ambon banana (*Musa paradisiaca L.*) has potential as an anti-inflammatory agent.

Keywords: anti-inflammatory, banana peel, red blood cell membrane

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1. Introduction

Inflammation is a localized protective response evoked by tissue damage caused by physical trauma, damaging chemical substances, or microbiological substances. Inflammation serves to destroy, reduce, or localize (sekuster) both damaging agents and damaged tissues [1]. Inflammation can be local and systemic, can also occur acutely or chronically causing pathological abnormalities. Treatment of inflammation includes two aspects, the first is to relieve pain which is often a symptom and the second is to stop the process of tissue damage. Reducing inflammation or the inflammatory response using steroids and non-steroidal anti-inflammatory drugs (NSAIDs) can actually relieve the inflammatory reaction well, but long-term use can have side effects in the form

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of decreasing endogenous glucocorticoid synthesis, decreasing the body's immune response to infections, osteoporosis, moonface and hypertension.

Currently, public interest in treatment with natural medicine is increasing. Utilization of plants both as medicine and other purposes is one of the phenomena that occurs today. Medicinal plants contain many components of active compounds and have various pharmacological effects that need to be scientifically proven. One of the medicinal plants that can be used for anti-inflammatory treatment is Ambon banana peel (*Musa paradisiaca L.*).

Therefore, the use of medicinal plants with anti-inflammatory properties needs to be done to find alternative treatments with relatively smaller side effects. Based on the results of research by Nifinluri, et al in 2019, It was found that the ethanol extract of the kepok banana peel with a dose of 75 mg/kgBB, 150 mg/kgBW and 300 mg/kgBW given orally, informalin-induced white mouse paws 1% respectively 47.39%, 59.53% and 81.50%. Ethanol extract treatment group with a dose of 300 mg/kgBW which shows the best inhibition compared to treatment group dose of 75 mg/kgBB and 150 mg/kgBB, it is known that the ethanol extract of the kepok banana peel has anti-inflammatory activity [2]. Research by Dewi, et al in 2021 with experimental study used 25 rats which were divided into 5 groups. Then each mouse paw was injected subplantar with 0.1 ml of 1% carrageenan suspension. After being measured, it was continued with the treatment provider topically according to the group. The positive control group was given diclofenac sodium gel, the negative control group was given the gel without the active substance, where the 15% banana stem extract gel group showed good results, where the 15% extract was effective in reducing inflammation in rat paws, this result can be seen from the almost comparable decrease in 15% inflammation of the extract gel with diclofenac sodium. stated that the Banana stem extract (*Musa paradisiaca L*) has the ability as a topical anti-inflammatory [3]. Inflammation is the body's reaction to the presence of infection, irritation or foreign substances as an effort of the body's defense mechanism. In the inflammatory reaction there will be the release of histamine, bradykinin and prostaglandins [4,5].

In addition to using the in vivo method based on the previous research, testing the anti-inflammatory activity of Ambon banana peel extract (*Musa paradisiaca L.*) can also be determined using the red blood cell membrane stabilization method, this is because the red blood cell membrane is analogous to the lysosomal membrane which can affect inflammatory process. Lysosomal membrane stabilization is important in limiting the inflammatory response, by preventing the release of lysosomal enzymes from granules in active neutrophils during the inflammatory process. Enzymes in neutrophils that are

released during inflammation (due to activation of neutrophils) will produce various disorders that can cause inflammation and further tissue damage. Stabilization of red blood cell membranes is a method that is widely used in research as a biochemical parameter for in vitro anti-inflammatory activity tests. The stability of red blood cells can be seen when red blood cells are induced with a hypotonic solution. The size of hemolysis that occurs in the red blood cell membrane induced by a hypotonic solution is used as a measure to determine anti-inflammatory activity [6].

Based on these problems, the purpose of this study was so important to know the anti-inflammatory activity of Ambon banana peel extract (*Musa paradisiaca L.*) using the red blood cell membrane stabilization method

2. Methods

2.1. Study design

This study design is a survey method with an experimental study in the Chemistry laboratory of the Faculty of health science and technology Jenderal Achmad Yani University in September 2021.

2.2. Sample

This study uses an experimental design with the Ambon banana (*Musa paradisiaca L.*) population obtained from the Cimahi market. Then the sample from this study was the Ambon banana (*Musa paradisiaca L.*) peel which was taken based on the criteria desired by the researcher, namely the skin of the fruit was a bit thick, 2.4-3 mm and yellowish in color, and not rotten nor damaged.

3. Measurement

3.1. Data Collection Procedure

This research begins with the manufacture of extracts with the extraction method used, namely the maceration method using absolute methanol as a solvent. Ambon banana peel (*Musa paradisiaca L.*) was macerated with absolute ethanol solvent in a ratio of 1:3. The residue was separated for 3 days, then filtered and the filtrate was obtained. The filtrate obtained was then concentrated with a rotary evaporator at room

temperature to obtain an ethanolic extract of Ambon banana peel (*Musa paradisiaca* L.). Furthermore, to determine the anti-inflammatory activity of the Ambon banana peel extract (*Musa paradisiaca* L.), in vitro testing was carried out using the red blood cell membrane stabilization method. The Anti-inflammatory Test Procedure was carried out by making a sterile Alsever solution, Phosphate Buffer pH 7.4 (0.15 M), Isosalin, Preparation of Red Blood Cell Suspension, Determination of Extract Concentration, Preparation of Extract Concentration and Diclofenac Sodium. Determination of the maximum absorption wavelength, and Measurement Extract Activity on Red Blood Cell Membrane Stabilization. The absorbance of the solution was measured on UV-Vis spectrophotometer at a wavelength of 560 nm. Each of the above solutions was then incubated at 37°C for 30 minutes and centrifuged at 5000 rpm for 10 minutes. The supernatant liquid obtained was taken and the hemoglobin content was calculated using a UV-Vis spectrophotometer at the maximum wavelength. The percent stability of the red blood cell membrane can be calculated by the formula, as follows:

$$\begin{aligned} & \% \text{ Test solution stability} \\ & = 100 - \left(\frac{\text{Abs Test Solution} - \text{Abs Test Control Solution}}{\text{Abs Negative Control Solution}} \right) \times 100 \% \end{aligned}$$

Anti-inflammatory activity was seen from the percentage of hemolysis that occurred in the addition of the extract. If the results are not much different from the standard, it indicates that the tested extract has anti-inflammatory activity.

Data Analysis.

The data obtained were analyzed descriptively by looking at the percentage (%) of Anti-inflammatory activity.

4. Results

The anti-inflammatory activity was tested using the in vitro human red blood cell (HRBC) membrane stabilization method. Measurements were made using a UV-Vis spectrophotometer at a wavelength of 560 nm because the absorbance of hemoglobin can be measured. The results of the sample ability in the HRBC test can be seen in Table 1.

Based on Table 1. Ambon banana peel extract (*Musa paradisiaca* L.) has potential as anti-inflammatory, this is evidenced by the percentage value of the stability of Ambon banana peel extract (*Musa paradisiaca* L.) in maintaining red blood cell membranes, which is 42.79% greater From the positive control, it can be seen that the higher the extract content, the higher its potential as an anti-inflammatory. At a concentration of 125 ppm the extract has the stability of maintaining the red blood cell membrane of

TABLE 1: Stability of the HRBC test to the peel extract of Ambon banana (*Musa paradisiaca L.*)

Concentration (ppm)	Test Solution Absorbance	Control Test Solution Absorbance	% Membrane stability
125	0,056	0,694	1,09
250	0,055	0,62	12,40
500	0,061	0,594	17,36
1000	0,077	0,446	42,79

1.09%, at a concentration of 250 ppm it has the stability of maintaining the red blood cell membrane of 12.40%, at a concentration of 500 ppm the extract has the same stability value as diclofenac sodium with a concentration of 500 ppm that is equal to 17.36%. At a concentration of 1000 ppm extract has a greater stability value than natrium diclofenac in maintaining red blood cell membranes, which is 42.79%. From these data, it can be concluded that Ambon banana peel extract (*Musa paradisiaca L.*) has potential as an anti-inflammatory seen from its high stability value and low hemolysis value in maintaining red blood cell membranes.

5. Discussion

The method used to determine anti-inflammatory activity in vitro is red blood cell membrane stabilization. This is because the red blood cell membrane is similar to the lysosomal membrane which has an influence on the inflammatory process, so the stabilization of the lysosomal membrane is important in limiting the inflammatory response, which is done by preventing the release of enzymes from the lysosomes during the inflammatory process. Enzymes in lysosomes that are released during the inflammatory process due to the activation of neutrophils will produce various disorders associated with acute or chronic inflammation. Therefore, the stability of red blood cell membranes against disturbances induced by hypotonic solutions can be used as a method to determine the stabilization of lysosomal membranes [7]. The stability of human red blood cells can be seen when red blood cells are induced by a hypotonic solution, causing a process of solvent transfer through a semipermeable membrane from a low concentration solution to a higher concentration solution and can cause lipid and protein oxidation, triggering membrane damage which is characterized by hemolysis occurs. The size of the hemolysis that occurs in the red blood cell membrane induced by the hypotonic solution is used as a measure to determine the presence of anti-inflammatory activity [6].

This Ambon banana peel extract (*Musa paradisiaca* L.) has anti-inflammatory activity which can be seen from the decrease in absorbance in the test solution. The smaller the absorbance value produced, the smaller the hemolysis that occurs, so the greater the anti-inflammatory activity possessed by the sample. Based on this principle, the anti-inflammatory activity of the Ambon banana peel extract (*Musa paradisiaca* L.) can be seen from the decrease in the absorbance value in the test solution and compared with the positive control. The anti-inflammatory activity of this extract is not only seen from the absorbance value but also needs to be calculated the percentage of inhibition of red blood cell lysis using the percentage stability formula. If the percentage value of the stability of the extract approaches or exceeds the positive control, it can be said to be strong because it has anti-inflammatory activity that is comparable to or more than the positive control. For positive control, natrium diclofenac is used because it is an anti-inflammatory drug belonging to the NSAID class that is widely used to treat inflammation and is easily available and can also inhibit the synthesis of prostaglandins or cyclooxygenase by preventing the release of anti-inflammatory mediators [8]. The yield of percent stability of the peel extract of Ambon banana (*Musa paradisiaca* L.) which has the highest anti-inflammatory activity is at a concentration of 1000 ppm, which is 42.79%. Judging from the results of the stability percentage, there was an increase in the concentration of the extract which indicated an increase in the potential of the extract in stabilizing the red blood cell membrane, so that its anti-inflammatory potential also increased.

Based on existing studies, it shows that with increasing levels or concentrations of an extract, its activity as a drug also increases. Therefore, compounds with membrane stabilizing activity are expected to provide protection to the lysosomal membrane in limiting the release of wound-causing substances [9]. Compounds with red blood cell stabilizing or lysosomal stabilizing properties are known for their ability to interfere with the early phase of the inflammatory reaction, namely the release of the enzyme phospholipase A2. Phospholipase A2 in cell membranes functions to convert phospholipids into arachidonic acid which is highly reactive and rapidly metabolized by cyclooxygenase (prostaglandin synthesis). Prostaglandins are the main components that cause pain and inflammation [7].

Normal red blood cells can also be seen through the stability of their membranes. When red blood cells are exposed to harmful substances such as hypotonic solutions or are in a heated medium, hemolysis will occur. In a hemolysis state, the cell membrane is in a state of injury so that the cell is more susceptible to free radicals which can lead to lipid peroxidation and will result in more severe cell damage [10].

6. Conclusions

Based on these results, it can be concluded that the peel of the Ambon banana (*Musa paradisiaca* L.) has potential as an anti-inflammatory agent. It is hoped that further research can be carried out on the potential of other parts of the banana plant that can be used as anti-inflammatory. And more research on the mechanism of reducing inflammation of secondary metabolites contained in Ambon banana peel (*Musa paradisiaca* L.)

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