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*Research Paper*

# Anti-Inflammatory Activity Test of Ethanol Extract of Ambarella Fruit Leaves (*Spondias dulcis Frost*) Against Male Rats Induced Carrageenan

***(Uji Aktifitas Anti-Inflamasi Ekstrak Etanol Daun Kedondong (Spondias dulcist Frost) pada Tikus Jantan yang Diinduksi Karagenan)***

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**Abstract:** In the treatment of inflammation, there are several chemical drugs, namely steroidal and non-steroidal anti-inflammatory drugs, but because they have many side effects, anti-inflammatory drugs are developed from natural ingredients, especially plants. Plants are scientifically proven to have anti-inflammatory properties, one of which is ambarella fruit leaves because it leaves contain flavonoid secondary metabolites as anti-inflammatory compounds. The objective of this study to determine the effect of giving ambarella fruit leaves on anti-inflammatory activity in male rats induced by carrageenan. The method used in this study was paw edema, namely by induction of carrageenan 0.1 ml as an irritant on the soles of the rat's feet for 360 minutes with the parameter observed was the inhibition of edema in the feet of rats using a plethysmometer. This study was an experimental study that was divided into 5 groups, namely the control group positive (diclofenac sodium 9 mg/kgBW), negative control (Na-Cmc 0.55), and three doses tested, namely 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW. The results of this study showed a significant difference ($p<0.05$) where flavonoid compounds from ambarella fruit leaves affect anti-inflammatory activity. The conclusion that the three doses is tested, namely 100 mg/kgBW, 300 mg/kgBW and 500 mg/kgBW has an anti-inflammatory effect where the more effective dose was 300 mg/kg kgBW.

**Keywords:** anti-inflammatory, ambarella fruit leaves, plethysmometer, carrageenan

**Abstrak:** Dalam penanganan inflamasi terdapat beberapa obat kimia yaitu golongan obat anti-inflamasi steroid dan non-steroid tetapi karena memiliki banyak efek samping sehingga dilakukan pengembangan anti-inflamasi yang berasal dari bahan alam terutama pada tanaman. Tanaman yang terbukti secara ilmiah memiliki khasiat sebagai anti-inflamasi diantaranya adalah daun kedondong karena mengandung senyawa metabolit sekunder flavonoid sebagai anti-inflamasi. Tujuan penelitian ini adalah untuk mengetahui pengaruh pemeberian daun kedondong terhadap aktivitas anti-inflamasi pada tikus jantan yang diinduksi karagenan. Metode dalam penelitian ini adalah dengan paw udem yaitu dengan induksi karagenan 0,1 ml sebagai iritan pada telapak kaki tikus selama 360 menit dengan parameter yang diamati adalah penghambatan udem kaki tikus menggunakan pletismometer. Penelitian ini adalah penelitian eksperimental yang dibagi menjadi 5 kelompok yaitu kontrol positif (Natrium diklofenak 9 mg/kgBB), kontrol negatif (Na-Cmc 0,55), dan tiga dosis yang diuji yaitu 100 mg/kgBB, 300 mg/kgBB, dan 500 mg/kgBB. Penelitian ini menunjukkan adanya perbedaan yang signifikan ($p<0,05$) dimana senyawa flavonoid dari daun kedondong memiliki pengaruh terhadap aktivitas anti-inflamasi. Kesimpulan: tiga dosis yang diuji yaitu 100 mg/kgBB, 300 mg/kgBB, dan 500 mg/kgBB memiliki efek anti-inflamasi dimana dosis yang lebih efektif adalah 300 mg/kgBB.

**Kata kunci:** anti-inflamasi, daun kedondong, pletismometer, karagenan

## 1. Introduction

Inflammation is a common occurrence and even has a fairly high incidence (Yuliati, 2010). Inflammation is a normal body defense response to tissue injury caused by physical trauma, damaging chemical substances, or microbiological substances (Mycek, 2001). Usually, conditions such as redness (rubor), heat (calor), swelling (tumor), pain (dolor), and impaired tissue function are signs of an inflammatory response (Corwin, 2008). Each type of response that occurs in inflammation causes discomfort for the patient so that appropriate treatment is needed in overcoming the signs of an inflammatory response for example by using (generic or patent drugs) drugs derived from plants or herbal plants (Supriyatta et al, 2015).

The treatment of inflammation, in general, can be done using modern drugs which have high side effects. At this time, various kinds of drugs are commonly used to treat inflammation or symptoms of the inflammatory response (Tjay and Rahardja, 2007).

Anti-inflammatory steroids or steroid anti-inflammatory drugs (SAID) for example can cause a decrease in immunity to infection, increased intraocular pressure, osteoporosis, atrophy of muscle and fat tissue, and are diabetes. Meanwhile, non-steroidal anti-inflammatory drugs (NSAIDs) can cause gastric ulcers to bleed, anemia, and kidney disorders (Anonymous, 2005). Therefore, it is necessary to develop and utilize traditional anti-inflammatory drugs derived from plants to be used as alternative treatments with fewer side effects than modern drugs (Kinanti, 2016). One of them is the ambarella plant (*Spondias dulcis Frost*) originating from South Asia, Southeast Asia and spread throughout the tropics. This is one of the plants that can be used as herbal medicine. This plant is believed by the community to have many benefits in the fruit and also the leaves. Some of the benefits of ambarella include treating dysentery, ulcers, burns, sore skin, and coughs. This is because the ambarella fruit plant (*Spondias dulcis Frost*) contains secondary metabolites, namely flavonoids, tannins, and saponins which have anti-bacterial, anti-histamine, anti-viral, anti-oxidant, anti-inflammatory, and anti-cancer properties (Harmanto, 2002).

The leaves, bark, and roots of the ambarella plant (*Spondias dulcis Frost*) contain flavonoid compounds, saponins, and tannins (Inayati, 2007). Saponins and tannins are predicted as anti-bacterial compounds in the leaves of ambarella fruit (*Spondias dulcis Frost*), besides that saponins are also efficacious as a trigger for collagen growth (Inayati, 2007). Ambarella fruit leaves (*Spondias dulcis Frost*) can also be useful as anti-fungals due to the activity of anti-fungal compounds, namely flavonoids, alkaloids, saponins, and tannins. The content of flavonoid compounds is the largest group of polyphenolic compounds in nature and acts as anti-oxidants (Frengki, 2007). Based on research Inayati 2007 show that plant leaves Ambarella (*Spondias dulcis Frost*) also has potential as an alternative herbal medicine to decrease cholesterol levels in the body because it contains flavonoids which are compounds such as polyphenol compounds, alkaloids, saponins, tannins, and vitamin C.

From the various results of the research that has been done, the content of chemical compounds which have anti-inflammatory properties are flavonoids (Ramadhani and Sri, 2017). Flavonoid compounds have inflammatory activity by inhibiting the release of serotonin and histamine to the site of inflammation, inhibiting the synthesis of prostaglandins from arachidonic acid by inhibiting cyclooxygenase (COX) (Hasanah, 2011). In addition to flavonoids other bioactive compounds that have the potential to an anti-inflammatory are saponins. The anti-inflammatory mechanism of saponins is by inhibiting the formation of exudate and inhibiting vascular permeability (Soemarie, 2016).

Based on the description above, the leaves of the ambarella plant (*Spondias dulcis Frost*) contain flavonoids which are expected to be used as new herbal medicines in anti-inflammatory treatment. The author conducted a study to determine the anti-inflammatory effect of extracts from ambarella fruit leaves on white male rats induced by 1% carrageenan.

## 2. RESULTS AND DISCUSSION

In this study, an anti-inflammatory or inflammatory test was carried out with the method used was paw edema or artificial swelling of the soles of the rat's feet using carrageenan as an inducer, where carrageenan is a foreign substance (antigen) which when it enters the body will stimulate the release of inflammatory mediators. This method was chosen because simple, easy to do, and often used. The use

of carrageenan as an inducer has several advantages, including not causing tissue damage, not leaving scars, and also providing a more sensitive response to anti-inflammatory drugs (Fitriyani, 2008).

Carrageenan as an irritant induces cell injury through the release of mediators that initiate the inflammatory process. At the time of the release of inflammatory mediators, edema is maximal and lasts several hours. Inflammation-induced by carrageenan is characterized by increased pain, swelling, and prostaglandin synthesis up to 4-5 times. Edema caused by carrageenan induction persists for 6 hours and gradually decreases within 24 hours (Taufiq, 2008).

Testing the anti-inflammatory activity of EEDK using 25 test animals, with 5 treatment groups. The group consisted of a positive control who was given diclofenac Naat a dose of 9 mg/kgBW orally, a negative control was given CMC Na 0.5% orally, an extract treatment group at a dose of 100mg/kgBW, an extract treatment group at a dose of 300 mg/kgBW, and the extract group at a dose of 500 mg/KgBW.

The rats have first fasted for ±18 hours, then the rats were weighed and marked on the tail and left ankle of the rats. Before each group was given ethanol extract of ambarella fruit leaves, the volume of the rat's feet was first measured as the initial volume (Vo). After that, each group was given an ethanolic extract of ambarella fruit leaves, namely group I was given 0.5% Na-CMC suspension, group II was given 9 mg/KgBW sodium diclofenac suspension, groups III, IV and V were each given 100 doses of EEDK suspension 500 mg/kgBW orally. One hour later, each mouse paw was injected intraplantar with 0.1 ml of 1% -carrageenan solution.

Measurements were carried out using a plethysmometer with the principle of measurement based on the *Archimedes'* law. After 30 minutes, measurements were made by dipping the mouse's paw into a plethysmometer cell containing a special liquid until the solution reached the upper limit line, and the pedal was held. Recorded numbers on the monitor. Changes in fluid volume are recorded as the volume of the rat's paws (Vt). Measurements were taken every 60 minutes for 360 minutes.

There are three phases of edema formation due to carrageenan induction, the first phase is the release of histamine and serotonin shortly after induction until 90 minutes after induction, the second phase is the release of bradykinin at 1.5 to 2.5 hours after induction, and the third phase is the release of prostaglandins that occur at 2.5 hours to 5 hours after induction (Di Rosa *et al.*,1971).

It can be seen that the percentage of inflammation in the five test groups increased continuously from the 60th minute to the 120th minute after carrageenan induction (Figure 1). This occurs because of the release of histamine, serotonin, and bradykinin in the tissue after carrageenan induction until the 120th minute. The largest inflammation percentage occurred at 120 minutes (Na.Cmc suspension) and was followed by 500 mg EEDK, 300 mg EEDK, Na suspension, diclofenac, and EEDK 100 mg. Meanwhile,

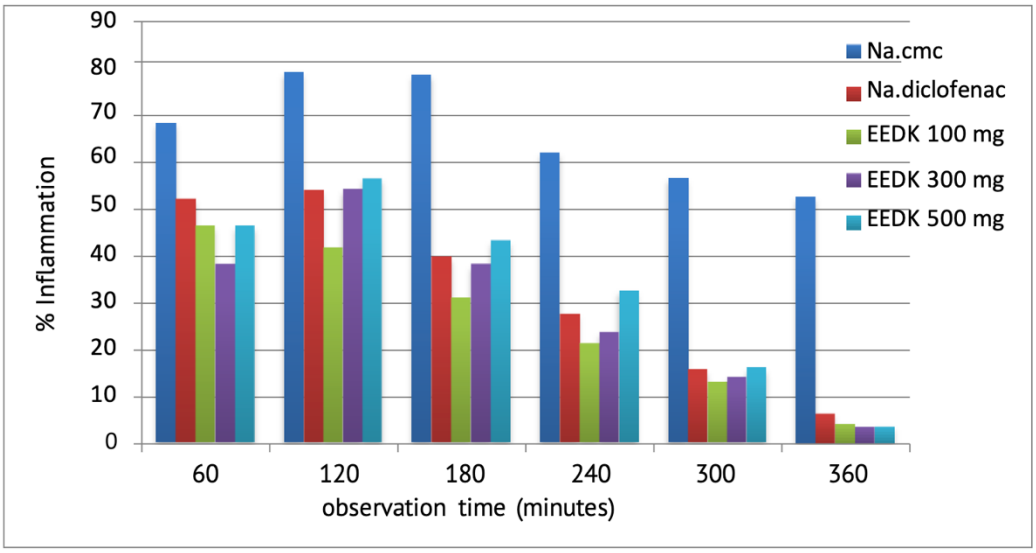


**Figure 1.** The average percentage of inflammation of the soles of mice

the Na.diclofenac group, as well as the EDK 100, 300, and 500 mg/kgBW, began to experience a decrease in the percent inflammation at the 180th minute, this was due to the inhibition of prostaglandins to the tissues by the four test groups. the Na. Cmc group began to experience a decrease and increase in the percentage of inflammation at the 180th minute to the 360th minute which was suspected to be inhibiting the release of prostaglandins by the body but not too strong compared to the test group.

Based on the results of the percentage of inflammation obtained, it showed that the four test groups, namely the Na.diclofenac group, EEDK 100, 300, and 500 mg/kgBW had an anti-inflammatory effect at the 180th minute to the 360th minute while Na.Cmc did not give this effect. The results of statistical analysis using SPSS with a 95% confidence level showed that the average fifth percent inflammation data, the average percent inflammation analyzed using *one-way* ANOVA with a 95% confidence level, showed that there was a significant difference (P<0.05) in the 180th minute to the 360th minute between theNa.Cmc group with Na.diclofenac, EEDK 100,300 and 500 mg/kgBW. In this case, it shows that the Na.diclofenac, EEDK 100, 300, and 500 mg/kgBW groups experienced a significant decrease in inflammation percentage from 180 minutes to 360 minutes, where the decrease in inflammation percentage was more significant or the smallest starting from 300 EEDK mg/kgBW, EEDK 100 mg/kgBW, Na.diclofenac, and EEDK 500mg/kgBW.

The percentage of foot inflammation in rats which was smaller than the control indicated that diclofenac sodium suspension and EEDK suspension of 100, 300 and 500 mg/kgBW were able to inhibit inflammation in rat paws caused by carrageenan. The ability to inhibit inflammation, which is called inflammation inhibition, can be seen in Figure 2.

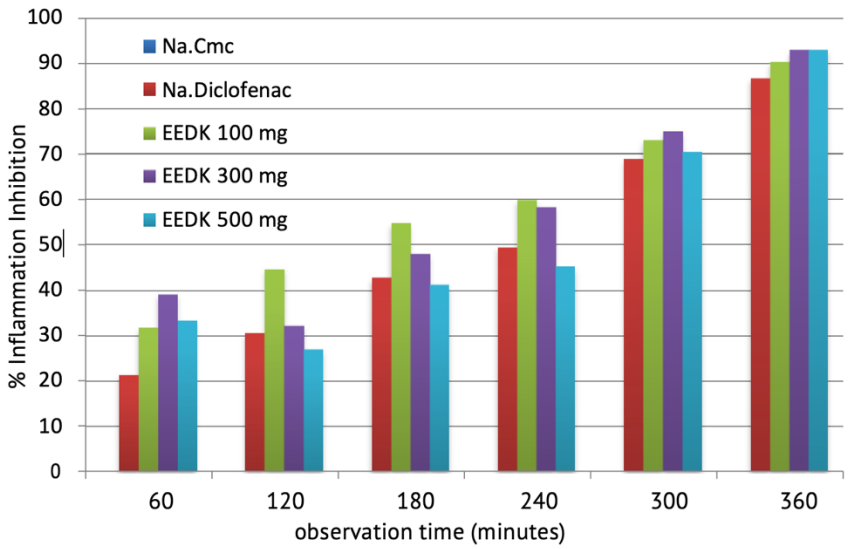


**Figure 2.** The percentage of average inflammation inhibition of the rat paws

It can be seen that the highest percentage value of inflammation inhibition was in the 300 and 500 mg/kg BW EDK group and the activity was better than the Na. 100 mg/kg body weight. This indicated that the Na.diclofenac, EEDK 100, 300, and 500 mg/kgBW groups had potential as anti-inflammatory agents while the group Na.Cmc did not (Figure 2). The results of statistical analysis using one-way ANOVA with a 95% confidence level showed a significant difference (P<0.05) between the Na.Cmc group and the Na.diclofenac group, EEDK 100, 300, 500 mg/kgBW. And showed no significant difference (P<0.05) between the Na.diclofenac group and the EEDK of 100, 300, 500 mg/kgBW. This means that the EDK group of 100, 300, 500 mg/kgBW has an anti-inflammatory activity that is comparable to the positive control or Na.diclofenac but in this study, the activity of the Na.diclofenac and EEDK 100 and 500 mg/kgBW group was weaker than that of the Na.diclofenac group EEDK 300 mg/kgBW. For this reason, the effective dose of ambarella fruit leaves extract in anti-inflammatory activity based on the results of this study is EEDK 300 mg/kgBW. Based on these test results, it can be concluded that the administration

of ethanol extract of ambarella fruit leaves at doses of 100, 300, and 500 mg/kg bw can reduce inflammation on the soles of male white rats induced by carrageenan.

## 3. Research Methods

The type of research used in this study is a pure experimental method. The stages of this research include sample preparation, sampling, preparation of experimental animals, simplicia characteristics, phytochemical screening, extraction methods, and testing of anti-inflammatory effects on white male rats. The basis of this method is to make edema (Paw Udem) on the back paws of mice using 1% carrageenan.

### *3.1. Tools and Materials*

The tools used in this research are laboratory glassware, aluminum foil, blender, saucer, incubator, digital camera, mouse cage, label, mortar and stamper, drying cabinet, microscope, analytical balance, oral sonde, oven, water bath, parchment, spatula, 1 ml and 3 ml suplantar, oral injection syringes, animal scales, rolled tissue, Ugobasille – Plethysmometer, Vacuum Rotary Evaporator, and Water bath.

The ingredients used in this study were distilled water, hydrochloric acid P, nitric acid, sulfuric acid P, iron (III) chloride P, bismuth nitrate, ambarella leaf extract, 96% ethanol, ether, ethyl acetate, iodine, isopropanol, potassium iodide, Carboxy Methyl Cellulose (CMC), chloroform, 0.9% NaCl solution, 0.9% sodium chloride solution, methanol, mercury (II) chloride, sodium diclofenac (PT. Indo Farma), Pb(II) acetate, magnesium powder, zinc powder, naphthol, and λ-carrageenan 1%.

### *3.2. Anti-Inflammatory Activity Test*

Before the test, rats have fasted for ±18 hours while still being given water. Rats were grouped into 5 groups: Group I: 0.5% Na-CMC suspension (negative control), Group II: diclofenac sodium suspension (positive control), Group III: EDK dose of 100 mg/kgbw, Group IV: EEDK dose 300 mg/kg bw, Group V: EEDK dose 500 mg/kgbw.

On the day of testing, each animal was weighed and marked on its left leg, then the left leg of the rat was put into a cell containing a reservoir solution that had been prepared previously until the liquid rose to the upper limit line, the pedal was then held, the number was recorded on the monitor as volume. Initial volume (Vo) is the volume of the foot before being given treatment. The negative control group was given CMC, the positive control group was given sodium diclofenac at a dose of 9 mg/kgbw and the other three groups were given the test material according to the planned dose orally. One hour later, each mouse paw was injected intraplantar with 0.1 ml of 1% carrageenan solution. After 30 minutes, measurements were taken by dipping the mouse's paw into a plethysmometer filled with a special liquid until the liquid reached the upper limit and the pedal was held. The numbers on the monitor for changes in fluid volume that occur are recorded as the volume of the rat's paws (Vt). Measurements were taken every 30-60 minutes for 6 hours. Each measurement of the cell solution was completed until the red line or red line at the top of the cell and at the main menu was pressed the 0 button, also the feet of the mice were dried previously.

The data obtained from the anti-inflammatory effect test were data on the percentage of inflammation and the percentage of inflammation inhibition on the soles of the rats' feet after being treated. Calculations can be done with the formula:

$$\%\ inflammation = \frac{Vt - Vo}{Vo} \times 100\%$$

where, $Vt =$ Volume of edema of the feet at t − time

$Vo =$ Initial volume of the rat′s feet

$$\%\ inflammation\ of\ Inhibition = \frac{a - b}{a} \times 100\%$$

where, $a =$ Percentage of the average inflammation of the negative control group

$b =$ Percentage of the average inflammation of the test material group and the positive control group

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