Anti-Inflammatory Activity Test fo Ethanol Extract of Kluwih Leaves (Artocarpus camansi Blanco) on Male Wistar White Rats (Rattus norvegicus)

Laela Suci Nofiyanti¹, Sunarti², Galih Samodra*³

¹⁰⁻³ Pharmacy Study Program, Faculty of Health, Harapan Bangsa University, Jl. Raden Patah No. 100, Purwokerto, Indonesia

*Corresponding author: galihsamodra@uhb.ac.id

ABSTRACT

Inflammation occurs when the body reacts to infection, irritation, or other damage. Some compounds in kluwih leaves are believed to have anti-inflammatory potential, one of which is flavonoids. The aim of this study was to find out the activity and effective dosage of the ethanol extract from kluwih leaves as an anti-inflammatory in male white Wistar rats. The experimental animals were divided into five groups: the negative control group (0.5% Na-CMC), the positive control group (Na-diclofenac), and the three treatment groups were given ethanol extract from kluwih leaves at doses of 175, 350, and 700 mg/KgBW orally. After 30 minutes, the soles of the rats' feet were induced with 0.1 ml of 1% carrageenan subplantarily. Edema volume was assessed every 1 hour for 6 hours using a plethysmometer. The data was statistically analyzed using One-Way ANOVA with LSD post hoc test. The research data shows that the ethanol extract of kluwih leaves at doses 175, 350, and 700 mg/KgBW has anti-inflammatory activity. The effective dose of kluwih leaf ethanol extract was 700 mg/KgBW (28.68%) with the second highest percentage of anti-inflammatory power value after the positive control group (31.75%). The LSD (Least Significant Difference) results indicate that the ethanol extract of kluwih leaf at a dose of 700 mg/KgBW does not show a significant difference in its anti-inflammatory effect with the positive control (0.442>0.05).

INTRODUCTION

Inflammation is the initial response to irritation, infection, or other forms of damage and is considered a protective agent to neutralize attacking agent and repair damaged tissue (Sumitra & Pasaribu, 2022). The common treatment or management used for inflammatory diseases is the group of nonsteroidal anti-inflammatory drugs (NSAIDs), which often have the potential to cause harmful side effects in the body (Khatami et al., 2022). One example of a commonly consumed nonsteroidal anti-inflammatory drug (NSAID) is sodium diclofenac, which has a strong inhibitory effect on cyclooxygenase in its anti-inflammatory action. This drug has the ability to inhibit the formation of prostaglandins as pain mediators and reduce the level of free arachidonic acid concentration in leukocytes by regulating the release and absorption of fatty acids (Novika et al., 2021).

Currently, the utilization of wild plants as a source of medicine is widely practiced by the community. Compounds found in plants have various health benefits. Although traditional treatment tends to be slower in healing compared to chemical drugs, it is believed to be safer due to minimal side effects and easy accessibility (Hilaliyah, 2021). A plant that can...
be employed as an alternative remedy is kluwih (Artocarpus camansi Blanco).

It is recognized that kluwih leaves contain phytochemical compounds like flavonoids, alkaloids, tannins, phenolics, saponins, triterpenoids, and glycosides (Sogandi & Amelia, 2020). Ethanol extract from kluwih leaves is known to have anti-hyperuricemia, antibacterial, SGPT (Serum Glutamic Pyruvic Transaminase) activity-reducing, and blood sugar-reducing properties. In the case of ethanol extract from breadfruit leaves (Artocarpus altilis) at a dosage of 500 mg/kgBW, it has been demonstrated to exhibit anti-inflammatory effects on edema in mice induced by CFA (Complete Freund’s Adjuvant). The research results after 28 days post-CFA injection showed that the edema volume in the ethanol extract of breadfruit leaves group (0.303±0.003 mm) and the K(+) group (0.283±0.023) was significantly smaller compared to the K(-) group (p<0.05) (Haqqi & Wahyuni, 2023; Nagara, 2019; Sogandi & Amelia, 2020; Widhiastuti et al., 2021).

Based on the explanation above, the aim of this study was to determine the activity and effective dosage of ethanol extract from kluwih leaves (Artocarpus camansi Blanco) as an anti-inflammatory agent in male white rats. Therefore, this study is expected to provide additional knowledge about the benefits of using ethanol extract from kluwih leaves (Artocarpus camansi Blanco) as an alternative in anti-inflammatory treatment.

**METHODS**

**Tools**

Glassware (Pyrex®), blender (Philip), analytical balance (Kenko KK-LAB), rotary evaporator (Biobase), waterbath (Memmert), reaction tube rack, syringe, stopwatch, and mercury plethysmometer (manual).

**Ingredients**

The required ingredients include fresh kluwih leaves obtained from Purwojati, Banyumas, male white rats, 96% ethanol (technical), aquadest (technical), carrageenan (Lansida®), Na CMC (Aloin®), 0.9% NaCl (Braun®), and sodium diclofenac tablets (Kimia Farma).

**Determination**

The determination of kluwih leaves (Artocarpus camansi Blanco) was conducted at the Faculty of Biology Environmental Laboratory, Jendral Soedirman University, to confirm the authenticity of kluwih leaves (Artocarpus camansi Blanco).

**Preparation of Simplisia**

Fresh kluwih leaves are wet-sorted, then thoroughly washed. Subsequently, the kluwih leaves are chopped and dried using sunlight, covered with cloth, and then dry-sorted to eliminate any damaged or dirty samples. The dried kluwih leaves are further finely ground using a blender and sifted through a mesh size 30 sieve (Artifah et al., 2022).

**Extraction Process**

One kg of powdered kluwih leaf simplicia (Artocarpus camansi Blanco) was weighed and macerated with 96% ethanol for 3x24 hours with stirring. The macerate was filtered, and afterward, the resulting filtrate was thickened with a rotary evaporator maintained at 50°C and evaporated with a water bath to obtain a concentrated extract. Afterward, the concentrated extract was weighed to calculate the yield (Sari et al., 2020).

**Phytochemical Screening**

**Alkaloids**

Two mL of ethanol extract from kluwih leaves was mixed with two mL of hydrochloric acid, heated for 5 minutes, and then filtered. After that, Mayer’s reagent was added in the amounts of three drops. The formation of a red, orange, or yellow color indicates the presence of alkaloid compounds (Rahmawati et al., 2023).

**Flavonoids**

Two mL of ethanol extract from kluwih leaves was mixed with a sufficient amount of hot water, then heated for 5 minutes and filtered. Five mL of the filtrate was taken, fifty mg of Mg powder and one mL of concentrated HCl were added, and then it was shaken vigorously. The formation of a red, orange, or yellow color indicates the presence of flavonoid compounds (Sami et al., 2017).

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Saponins

Ten mL of hot water was added to five hundred mg of ethanol extract from kluwih leaves. After that, cool it down and shake for 10 seconds. Saponin compounds are characterized by the formation of a stable foam that forms for at least 10 minutes, and if 2N HCl is added, the foam does not disappear (Rusli et al., 2022).

Tannins

Three drops of FeCl$_3$ were mixed into two mL of ethanol extract from kluwih leaves. A dark blue or blackish-green color indicates the presence of tannin compounds (Prayogi et al., 2022).

Steroid-triterpenoids

Chloroform was added to the ethanol extract of kluwih leaves and then filtered. 1 ml of anhydrous acetic acid was added to the filtrate, heated, and then cooled. Subsequently, concentrated H$_2$SO$_4$ was added. A brown or violet ring indicates the presence of triterpenoid compounds. Meanwhile, the formation of a bluish-green color indicates that the extract contains steroids (Takaeb & Leo, 2023).

Preparation of test animals

Rats were acclimatized for approximately 1 week, ensuring their food and water needs were met. Subsequently, the rats underwent fasting for 18 hours before the experiment, but they were still provided with drinking water (Santi et al., 2017). Each rat was marked with a marker on the hind leg so that when the rat's leg was placed in the plethysmometer, the mercury water level was the same for all. The experimental animals used were male Wistar white rats, weights ranging from 150-200 grams and ages between 2-3 months, and in good health. There were a total of 25 rats used, were divided into 5 groups, each group consisting of 5 rats. The number of rats used was determined based on the result of the Federer formula: (n-1) (t-1) ≥ 15, in this equation, ‘t’ is the number of treatments, and ‘n’ is the number of repetitions for each treatment (Purba et al., 2022).

Preparation of 0.5% Na-CMC solution

Five hundred mg of Na-CMC was added to 50 mL hot distilled water. It was stirred until a transparent mass was formed and then diluted with a small amount of distilled water. The mixture was transferred to a 100 mL measuring flask, and distilled water was added until it reached the marked line (Lallo et al., 2020).

Preparation of 1% carrageenan suspension

One gram of carrageenan was dissolved in 50 mL of 0.9% NaCl, homogenized, after that the volume was increased to 100 mL (Maulana et al., 2020).

Preparation of sodium diclofenac suspension

The dosage of sodium didclofenac 50 mg was calculated using a conversion factor from human to rat (200 g), which is 0.018. Therefore, the dosage of sodium diclofenac for rats is 0.9 mg/200 g BW (body weight) and it was dissolved in a 0.5% Na-CMC solution (Cahyaningsih et al., 2018).

Anti-inflammatory testing

Rats in each group, which were marked on their paws, had the normal paw volume of rats (Vo) measured by placing the right hind paw of each rat in a plethysmometer containing mercury. Each group received the test substance orally according to the prescribed dosage as follows:

- Group 1: 0.5% w/v Na-CMC (negative control)
- Group 2: Na-diclofenac (positive control)
- Group 3: ethanol extract of kluwih leaves 175 mg/kgBW
- Group 4: ethanol extract of kluwih leaves 350 mg/kgBW
- Group 5: ethanol extract of kluwih leaves 700 mg/kgBW

Subsequently, 30 minutes later, each treatment group was induced through subplantar administration with 0.1 mL of 1% carrageenan. Measurements were taken at one-hour intervals for 6 hours. Changes in the rat paw volume were recorded as the rat paw volume at time t (Vt). Then, the rat paw edema volume was calculated, followed by the calculation of the AUC (Area Under the Curve) and % anti-inflammatory power (Fitriyanti et al., 2020).

Data Analysis

Data were statistically analyzed using SPSS software. To assess the normality of the data, the Shapiro-Wilk test was used, and the homogeneity of the data was assessed using the Levene test. Data is considered homogeneous if...
the significance level is >0.05. If the data shows a normal distribution and homogeneous, then the analysis continues with the One-Way ANOVA test at a 95% level of confidence to determine whether there is a significant difference. If a significant difference is found, a Post Hoc test is conducted using the LSD method to see the differences between the treatment groups (Sunarti & Octavini, 2023).

RESULT AND DISCUSSION

The process of preparing ethanol extract from kluwih leaves was carried out by soaking kluwih leaf powder in 96% ethanol solvent for 3x24 hours using the maceration method. The selection of 96% ethanol as the solvent is because it possesses universal, selective, polar, non-toxic, readily available, good absorption properties, and high analytical capabilities, allowing it to extract various compounds with polar, semi-polar, and nonpolar properties. 96% ethanol can easily penetrate the cell walls of the sample compared to lower concentration ethanol and it evaporates easily, making it easier to obtain a concentrated extract (Wendersteyt et al., 2021).

After macerating for 3 days, the sample was filtered to get the filtrate. The filtrate was then concentrated in a rotary evaporator set at 50°C and evaporated on a water bath using a steam cup to remove the remaining ethanol in the extract, resulting in a thick, dark greenish-black kluwih leaf extract. The use of a 50°C temperature in the ethanol evaporation process is more efficient and faster, as at this temperature, ethanol is under vacuum conditions, making it easier to evaporate. Evaporation is carried out below the boiling point (<60°C) to avoid pressure that can cause solvent vapor to condense and then fall into the collection tube, thus preventing the compounds separated from the ethanol solvent from being damaged due to high temperature (Wardaniati & Yanti, 2018).

The yield of kluwih leaf ethanol extract obtained is 10.112%. This percentage yield is higher compared to the yield of kluwih leaf ethanol extract in Dewi et al., (2022) study, which was 4%. The yield obtained also aligns with the standards listed in the Indonesian Herbal Pharmacopoeia, 2nd edition, which specifies that the yield of concentrated breadfruit leaf extract should be >9.9% (Kementerian Kesehatan RI, 2017). Factors that can influence the yield value of an extract include the duration of the extraction, particle size of the sample, storage conditions, solvent type, and the proportion of the sample to the solvent (Wijaya et al., 2018).

Phytochemical screening aims to describe the groups of compounds present in a plant under study. Phytochemical screening methods can involve color reaction testing using color reagents (Islami et al., 2022). Below are the results of the phytochemical screening of ethanol extract of kluwih leaves (Table 1).

<table>
<thead>
<tr>
<th>Identification</th>
<th>Reagents</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer</td>
<td>cloudy yellow color</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Mg+HCl 2 N</td>
<td>yellow color</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>HCl 2 N</td>
<td>stable foam of 2.5 cm</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>FeCl3</td>
<td>blackish-green color</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>Lieberman-Burchard</td>
<td>brownish ring</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: (+) Presence of compound groups (-) Absence of compound groups

The outcomes of the aforementioned phytochemical screening reveal that the ethanol extract obtained from kluwih leaves contained various constituents like flavonoids, alkaloids, saponins, tannins, and triterpenoids. This is consistent with previous research where kluwih leaf extracts were found to contain compounds like flavonoids, alkaloids, saponins, tannins, and triterpenoids (Sogandi & Amelia, 2020).

The method used for the anti-inflammatory test is the induced swelling in the paw of male white rats using 1% carrageenan. The process of swelling caused by carrageenan consists of three stages. The first stage involves the release of serotonin and histamine and lasts for 90 minutes. The second stage, involving the release of bradykinin, takes place between 1.5 to 2.5 hours following the induction. Meanwhile, the third stage is the release of prostaglandin occurring 3 hours after the induction. Edema will rapidly reach its maximum size and will persist for approximately 6 hours after induction (Samodra & Kusuma, 2021).

As seen in Table 2, the negative control group is the group with the least reduction in effect, as it has the highest AUC value compared

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to the other groups. Meanwhile, the positive control group is the group with the highest reduction effect, as it has the smallest AUC value. The lower the AUC value, the smaller the edema formed, and the shorter the healing time (Marampa et al., 2022). This is because sodium diclofenac exerts its anti-inflammatory effect through inhibition of the formation of cyclooxygenase (COX), preventing the production of prostaglandins (Nurhidayati, 2020). In the LSD test, the AUC values also indicate a significant difference between the AUC values of the ethanol extract of kluwih leaves groups at 175, 350, and 700mg/KgBW with the negative control group. This suggests that the ethanol extract of kluwih leaves exhibits anti-inflammatory activity.

Meanwhile, the results of the % anti-inflammatory power in Table 2 show that the highest % anti-inflammatory power is in the positive control group at 31.75%, and the lowest % anti-inflammatory power is in the ethanol extract of kluwih leaves group at 175 mg/KgBW at 10.71%. The larger the % anti-inflammatory power, the greater the anti-inflammatory effect. % anti-inflammatory power is inversely related to the AUC value, where if the AUC value is large, the resulting % anti-inflammatory power is small, and vice versa (Apridamayanti et al, 2018).

The result of the LSD % anti-inflammatory power test, it is known that the positive control group and the ethanol extract of kluwih leaves 700 mg/KgBW group showed no significant difference in providing anti-inflammatory effects on the rat paw edema with a significance value of 0.442 (>0.05). This indicates that the ethanol extract of kluwih leaves 700 mg/KgBW group is an effective dosage of ethanol extract of kluwih leaves. This is because kluwih leaves contain compounds that can work as anti-inflammatories, including flavonoids, alkaloids, tannins, saponins, and triterpenoids. Flavonoids have anti-inflammatory activity by inhibiting the inflammatory process through two mechanisms: inhibiting capillary permeability and arachidonic acid formation and reducing the release of lysosomal enzymes in neutrophils and endothelial cells, thus inhibiting the proliferation and exudation of inflammation (Samodra & Kusuma, 2021).

Alkaloids exhibit anti-inflammatory effects by suppressing the release of histamine from mast cells and reducing the production of interleukin-1 by monocytes (Dwitiyanti et al., 2022). Saponins in anti-inflammatory activity are believed to interact with lipid membranes such as phospholipids, which play a role in the initial production of prostaglandins and other inflammatory mediators. Saponins also have the potential to inhibit the formation of exudates and vascular permeability (Astika et al., 2022; Batmomolin et al., 2022). Tannins in anti-inflammatory activity work by suppressing the production of oxidants by monocytes, macrophages and neutrophils (Dwitiyanti et al., 2022). Meanwhile, triterpenoids function by inhibiting the action of cyclooxygenase enzymes, which convert arachidonic acid into prostaglandins as inflammatory mediators (Muhammad et al., 2021).

**CONCLUSIONS**

From the research results, it can be concluded that all doses of ethanol extract from kluwih leaves administered demonstrate anti-inflammatory activity. The effective dose of kluwih leaf ethanol extract as an anti-
inflammatory agent in white rats is 700 mg/KgBW, with an anti-inflammatory activity percentage of 28.68%, comparable to Nadviklofenac at a dose of 0.9 mg/200gBW (0.442>0.05).

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AUTHORS’ CONTRIBUTIONS

All authors contributed equally to conducting this research.

CONFLICT OF INTERESTS

There is no conflict of interest in conducting this research.

ETHICAL CONSIDERATION

This research has been approved by the Health Research Ethics Committee of Harapan Bangsa University with the number B.LPPM-UHB/2023/07/2023.

BIBLIOGRAPHY


