Introduction

Inflammation is a normal protective response to tissue injury caused by physical trauma, damaging chemicals, microbes, or radiation. Inflammation is the body’s attempt to inactivate invading organisms, eliminate irritants, and regulate tissue repair [1]. Signs of inflammation can include redness (rubor), heat (calor), pain (dolor), and swelling (tumor). It is stated that most diseases cause symptoms of pain, which are then manifested in the form of pain in the organs or tissues of the body. Pain can also be a sign that something is not right in the body and can make it easier to diagnose a disease by looking at the nature and location of the occurrence [2].

Usually, inflammation is treated using steroid anti-inflammatory drugs (SAID) and non-steroidal anti-inflammatory drugs (NSAIDs). This class of drugs is widely used in the community because of their quick effect in eliminating inflammation, but they also have the risk of dangerous side effects [3]. Several harmful side effects arise from using NSAIDs and NSAIDs, such as impaired kidney function, edema, hypertension, and gastrointestinal bleeding [4]. Therefore, looking for alternatives by developing new drug compounds to control inflammation with relatively minor side effects is necessary.

One plant with anti-inflammatory potential is pucuk merah (Syzygium myrtifolium Walp.). This plant contains flavonoid, phenolic, and terpenoid compounds [5]. According to Liniawati et al. [6], pucuk merah leaves have antioxidant activity because they contain cyanidin-glycoside compounds. The 96% ethanol extract of pucuk merah leaves is efficacious as an antidiarrheal at 6.72 mg/20 gBW, equivalent to loperamide HCl [7]. Ethanol extract of pucuk merah leaves at a concentration of 6% is effective in healing burns in male white rats [8]. Another benefit of pucuk merah leaves is that they are an antibacterial and immunostimulant.

This study aimed to determine the analgesic and anti-inflammatory activity of a 96% ethanol extract of pucuk merah.
leaves collected from Sei Simpang Dua Village, Kampar Kiri Hilir District, Riau Regency, Indonesia. Knowing these plants’ analgesic and anti-inflammatory activity can optimize treatment that can be applied to the community by minimizing side effects due to the long-term use of synthetic drugs. The anti-inflammatory effect was tested by measuring the volume of rat leg edema induced by 1% carrageenan.

**Method**

**Determination Sample**
To confirm the type, leaf samples were identified at the Andalas University Herbarium (ANDA), Limau Manis, Pauh District, Padang City, Indonesia, and the samples were identified as *Syzigium myrtifolium* Walp.

**Tools and Materials**
The tools used in this research were glassware, mesh 30 sieve, dark glass bottle, steam cup, porcelain crucible, grinder, sonde needle, rat cage, mortar and stamper, mask, analytical balance (LabPRO®, Indonesia), oven (Memmert®, Germany), stainless knife, pletismometer (Ugo Basile, Italy), rat cage, mortar and stamper, mask, analytical balance (LabPRO®, Indonesia), oven (Memmert®, Germany), stainless knife, pletismometer (Ugo Basile, Italy), rat cage, mortar and stamper, mask, analytical balance (LabPRO®, Indonesia), oven (Memmert®, Germany), stainless knife, pletismometer (Ugo Basile, Italy), rat cage, mortar and stamper, mask, analytical balance (LabPRO®, Indonesia), oven (Memmert®, Germany), stainless knife, pletismometer (Ugo Basile, Italy), rat cage, mortar and stamper, mask, analytical balance (LabPRO®, Indonesia), oven (Memmert®, Germany), stainless knife, pletismometer (Ugo Basile, Italy), rat cage, mortar and stamper, mask.

The materials used in this research were distilled water, 2 N hydrochloric acid, iron (III) chloride (Merck®, Germany), CMC-Na 0.5%, pucuk merah leaves, 96% ethanol, gelatin (Merck®, Germany), 30 male white rats (*Rattus norvegicus*) with body weight ± 200–250 g, rat food, husks, batis cloth, a combination of sodium chloride gelatin 10%, hydrochloric acid solution P (HCL) (Merck®, Germany), carrageenan (sigma®, USA) solution 1%, solution ferric chloride 3%, 10%, sodium diolofenac (Hj, Indonesia), physiological sodium chloride 0.9%, Bouchardat's reagent, Dragendorff's reagent, Mayer’s reagent, and magnesium (Mg) powder (Merck®, Germany).

**Ethical Clearance**
Before testing anti-inflammatories using experimental animals, the research design was reviewed by the Ethics Committee for the Use of Experimental Animals, Faculty of Medicine, Riau University, to obtain approval for the Code of Ethics for treating test animals.

**Sample Preparation**
A sample of 4 kg of pucuk merah leaves was collected, wet-sorted, washed, and drained. Then, it is chopped and dried in an oven at 50–55 °C for three days. After drying, the impurities are removed, mashed, sifted using a mesh number 60 sieve, and stored in a tightly closed container. Simplesia obtained as much as 2 kg of simpicia powder, which was put into the maceration vessel and added to 96% ethanol solvent. The ship was closed tightly for 6 hours while stirring occasionally, then let stand for 18 hours. Then, it is filtered, and the filtrate is separated and poured in to separate the material that passes during filtering. The residue from the first maceration was then macerated again two times with the remaining solvent with the same treatment. The macerated filtrate was collected and evaporated using a rotary evaporator and then placed over a water bath to obtain a thick extract.

**Qualitative Biochemical Assays**

**Flavonoid**
Simpicia or extract was weighed at 0.5 g, and 5 mL of 95% ethanol was added. Then, take 2 mL, add 0.1 g of magnesium powder, and add ten drops of HCl P from the side of the tube. Shake gently; if a red or orange color forms, it indicates the presence of flavonoids.

**Saponin**
Simpicia or extract was weighed at 0.5 g and shaken with 10 mL of water (if necessary in a water bath). A positive reaction is indicated by stable foam that does not disappear when hydrochloric acid is added.

**Alkaloid**
Simpicia/extract was dissolved in several drops of 2 N sulfuric acid, stirred, and tested with alkaloid reagents: Mayer's reagent, Dragendorff's reagent, and Bouchardat's reagent. Positive results were shown in Mayer’s reagent; a white precipitate was formed; in Dragendorff's reagent, a red to orange residue was formed; and in Bouchardat's reagent, a yellowish brown precipitate was formed.

**Tanin**
The extract is extracted with ethanol, filtered, and the filtrate is taken. To a test solution, 10% gelatin is added; if it contains tannin, a white precipitate solution is formed. To the following sample, NaCl-gelatin is added to the test solution (1% gelatin solution in 10% NaCl solution); if it contains tannin, a white precipitate solution is formed. A few drops of 3% FeCl₃ were added to the sample. If the solution includes a blue-black color, it contains hydrolyzed tannins; if a blue-green color forms, it contains condensed
tannins.

**Triterpenoids dan Steroids**

0.1 g of extract was added with three drops of acetic anhydrous solution and one drop of concentrated H2SO4. Positive results are shown in red (triterpenoids) and green (steroids).

**Preparation of Diclofenac Sodium Suspension**

This study used a positive control of diclofenac sodium 50mg/KgBB. 50 mg diclofenac sodium was suspended with 0.5% CMC. CMC was sprinkled into hot water seven times until dissolved and homogeneous. Then, diclofenac sodium is added to the CMC mixture until it is evenly dispersed, and the remaining hot water is added to the desired volume [9].

**Ethical Clearance**

The Ethics Committee has reviewed the design of this study for the Use of Experimental Animals, Faculty of Medicine, Riau University, Riau Province, Indonesia. The Ethics Code has approved it with NO: B/062/UNI 9.5.1.1.8/UEPKK/2023.

**Animal Test Protocol**

A total of 25 healthy male Wistar rats were weighed and kept in plastic cages. The experimental rats were acclimatized for seven days in order to adapt to their new environment. Then, it was considered again to be randomly grouped with a total of 5 rats per group. Test animals were divided into five treatment groups, namely:

- **Group 1 Negative control (Na CMC 1%; 3.125 ml)**
- **Group 2 positive control (Na Diclofenac 50mg/KgBB as much as 0.22 ml)**
- **Group 3 (dose of pucuk merah leaf extract 75 Mg/Kg BB as much as 1.875 ml)**
- **Group 4 (dose of pucuk merah extract 100 mg/kg bw as much as 2.5 ml)**
- **Group 5 (dose of pucuk merah extract 125 mg / kg b as much as 3.125 ml)**

**Anti-inflammatory Activity Testing**

The initial paw volume of rats was measured using a plethysmometer before treatment and expressed as the initial paw volume (V0). All rats were given the test solution orally according to the predetermined dose. After 15 minutes, the rats were induced with 1% carrageenan suspension as much as 0.1 mL intraplantar. The rats were measured using a plethysmometer at the 30th, 60th, 120th, 150th, and 180th minutes, then expressed as the final volume (Vt). The percentage value of udem can be calculated using the formula [10]:

$$\left(\frac{V_t - V_0}{V_0}\right) \times 100\%$$

Description: $V_0$ = Initial udem volume  
$V_t$ = Final udem volume

The average percentage of inflammation inhibition was calculated using the formula [11]:

$$\% \text{ Inflammation inhibition} = \left(\frac{a-b}{a}\right) \times 100\%$$

Description:

$\ a = $ Average negative control group inflammation  
$\ b = $ Inflammation of the average treatment group

**Table 1. Phytochemical screening test results of pucuk merah plants (Syzygium myrtifolium Walp.)**

<table>
<thead>
<tr>
<th>No</th>
<th>Secondary Metabolite Testing</th>
<th>Simplicia</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Mayeir</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Drageindorf</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bouchardat</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Saponin</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Flavonoid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Triterpenoid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Steroid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Tannin</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>
The anti-inflammatory potential of pucuk merah extract was carried out by comparing the average inflammation inhibition of each dose treatment with the positive control, namely diclofenac sodium.

Data Analysis

The research design used in this research is an analysis of variance for a completely randomized design (RAL) because, in this experiment, there is only one factor to be observed, namely the dose factor. The data obtained were analyzed with the Kolmogorov-Smirnov test to see the distribution of data and analyzed with the Levene test to see the homogeneity of the data. If the data is usually distributed and homogeneity, then proceed with a one-way Analysis Of Variance (ANOVA) test. If the test requirements (ANOVA) are not met, the Kruskal Wallis and Tukey tests are continued to see whether there are differences between pairs of treatment groups [12].

Results and Discussion

Extraction Results

The results of making simplistic from 4 kg of wet leaves obtained 2 kg of pucuk merah leaf powder (50% yield). From organoleptic observations, the puck merah leaf simplistic powder was obtained in the form of coarse powder, red in color, had an aromatic smell, and had a slightly bitter taste. A thick extract of pucuk merah leaves was obtained through the maceration method using 1 kg of simplistic powder in 10 L of 96% ethanol solvent. 96% ethanol is used because it is a universal solvent that can attract compounds contained in pucuk merah, especially oleoresin compounds (Wahyuningtyas, 2017). The thick extract of pucuk merah leaves was obtained as much as 47.593 g (4.75% extract yield). Determination of work is done to determine the ratio between the amount of extract received and the amount of initial simplistic.

Qualitative Biochemical Assays Results

Simplisia and extracts of pucuk merah leaves contain flavonoids, saponins, alkaloids, triterpenoids, and steroids (table 1).

Phytochemical Test Results

Simplicia and pucuk merah leaf extract contain flavonoids, saponins, alkaloids, triterpenoids, and steroids (table 1).

Results of Anti-inflammatory Activity Testing

The anti-inflammatory activity of a drug is indicated by its ability to reduce udem induced in rat feet. It is said that a substance has an anti-inflammatory effect. If the test animal is caused by carrageenan, there is a decrease in swelling (percentage of inflammation inhibition) by 50% or more [13]. Diclofenac sodium is used as a positive control because it is rapidly absorbed after oral administration and has a short half-life; its wide use as an anti-inflammatory, Na diclofenac has activity by inhibiting the enzyme cyclooxygenase so that prostaglandin formation is inhibited [14].

Measurement of rat paw volume with a plethysmometer can be influenced by several factors, including the difficulty of conditioning test animals and clarity at the time of scale reading. This can be reduced by calming the test animals when inserting their feet into mercury, providing clear boundaries with permanent markers that are not easily lost, and taking measurements in triplicate for each test animal.
Measurement of udder volume was carried out for 3 hours, namely at the 30th, 60th, 90th, 120th, 150th, and 180th minutes, to see the increase in paw volume of the test animals after carrageenan induction. Figure 1 shows the rise in udder volume that is different between treatment groups. The increase in udder volume in the negative control was further from the other test groups. The negative control group was only given 1% Na-CMC orally and then induced with carrageenan. The udder volume of the negative control group increased from the 30th minute to the 120th minute; this was due to the absence of udder inhibitory activity by Na-CMC. Carrageenan, with a concentration of 1%, is an excellent idea-inducing agent and can cause significant inflammation. The role of carrageenan in generating edema is by stimulating inflammatory mediators such as histamine, serotonin, bradykinin, prostaglandins, and leukotrienes.

The release of inflammatory mediators by carrageenan is divided into three phases. In the first phase, carrageenan stimulates the release of serotonin and histamine during the first 1 hour, resulting in increased vascular permeability. Another inflammatory mediator, kinin, is released at 2 hours after induction (second phase). The final step at 2.5-3 hours after induction is the release of prostaglandins, which are closely related to the migration of leukocytes at the inflammatory site, causing udder. Acute inflammation caused by carrageenan will last for 5-6 hours; the role of free radicals in the development of carrageenan-induced acute inflammation has also been proven [15, 16].

The positive control group and groups 3, 4, and 5, namely doses 1, 2, and 3, experienced an increase in udder volume at the 30th minute and decreased at the 120th minute. From the udder volume data, the percentage value of udder can be calculated, which describes the amount of udder formed on the soles of rat feet after carrageenan induction. High udder volume is proportional to the ability of the test compound to inhibit udder formation. In testing anti-inflammatory activity, the value of udder stimulates the release of serotonin and histamine during the first 1 hour, resulting in increased vascular permeability. Another inflammatory mediator, kinin, is released at 2 hours after induction (second phase). The final step at 2.5-3 hours after induction is the release of prostaglandins, which are closely related to the migration of leukocytes at the inflammatory site, causing udder. Acute inflammation caused by carrageenan will last for 5-6 hours; the role of free radicals in the development of carrageenan-induced acute inflammation has also been proven [15, 16].

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inhibition produced by the test compound is called the percent inhibition of udem (inflammation).

The greater the anti-inflammatory power, the greater the ability to inhibit damage that occurs. Based on the calculation of the percentage of inflammation inhibition, the test group that has the most significant share of inhibition is group 5, which is 68% with an anti-inflammatory potential value of 97%, followed by group 4, which is 57% with an anti-inflammatory potential value of 81%. The inhibition of udem in group 5 with a dose of 125 mg/kg bw pucuk merah leaf extract is almost the same as the positive control of diclofenac sodium, which has an anti-inflammatory potential value of 100%. Group 3 has the smallest percent of inhibition, which is 44%, with an anti-inflammatory potential value of 62% (Table 2). In accordance with the theory presented earlier, the results of research with exudate volume parameters obtained from test doses of groups 4 and 5 have the potential as anti-inflammatory because they show a percentage of inhibition of more than 50%.

Data normality test with Kolmogorov-Smirnov showed that the data of all treatment groups were not normally distributed, so they did not qualify for the ANOVA test. Furthermore, the research data were tested with Kruskal Wallis and Tukey tests to see meaningful data [17].

Statistical test results showed a significant difference between the positive control group and group 3 (75 mg/KgBB). The statistical test results showed no significant difference between dose group 4 (100 mg/KgBB) and dose group 5 (125 mg/KgBB), with the positive control group at the 0.05 test level (p≥0.05). This proves that dose groups 4 and 5 have an anti-inflammatory effect equivalent to the positive control so as to reduce the volume of inflammation of the rat's foot caused by subplantar administration of carrageenan (table 2).

Several previous studies on plants conducted by Apridamayanti [18], Komakech [19], Adryan [20], and Dwitiyanti [12] showed that flavonoid phytochemical content is thought to have anti-inflammatory activity. Past research has shown that flavonoids activate antioxidant pathways that provide anti-inflammatory effects, inhibit the secretion of enzymes such as lysozyme and β-glucuronidase, and inhibit the secretion of arachidonic acid, which reduces inflammatory reactions [21]. Calculation of antibiotic potency (table 2) in percent of pucuk merah extract can be seen that the best power is given by dose groups 4 and 5 by 81% and 97% compared to the positive control, namely diclofenac sodium.

Conclusion

The most effective dose group as an anti-inflammatory was group 5, with a dose of pucuk merah leaf extract of 125 mg/kgBW with an inflammation inhibition percentage of 68% and an anti-inflammatory potential value of 97% compared to the positive control, namely diclofenac sodium.

Acknowledgment

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Reference


