



Anti-inflammatory Activity Test of Pacing Putih Flower (*Costus speciosus* (J. Koenig) Sm.)

Madaniah Fathirah^{1(✉)}, Eva Marlina^{1,2}, and Ritbey Ruga^{1,2}

¹ Department of Chemistry, Mulawarman University, Samarinda, Indonesia
mada.fathirah34@gmail.com

² Center of Excellence for Science and Technology-College of Medicine and Cosmetics From Humid Tropical Forests and Their Environment (PUI-PT OKTAL), Mulawarman University, Samarinda, Indonesia

Abstract. The anti-inflammatory activity of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was tested. This study aims to determine the anti-inflammatory activity of the ethyl acetate fraction and methanol-water fraction. The method used was inhibition of protein denaturation using bovine serum albumin and positive control, namely sodium diclofenac. The ethyl acetate fraction contains steroid and quinones. The methanol-water fraction contains phenolics and flavonoids. Anti-inflammatory activity (IC₅₀) on ethyl acetate fraction and methanol-water fraction, respectively, were 80.1728 and 14.9772 mg/L. Ethyl acetate fraction and methanol-water fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) have potential to have anti-inflammatory activity.

Keywords: (*Costus speciosus* (J. Koenig) Sm.) · Anti-inflammatory · Protein Denaturation

1 Introduction

Inflammation is the basic process by which body tissues respond or react to infection, irritation, or other injuries. Inflammation is defined by the presence of 5 macroscopic pathological phenomena, namely tumor (swelling of tissue), calor (increased tissue temperature), rubor (redness of the vascular tissue), dolor (painful stimulation of the tissue), and functio laesa (impaired function of the affected organ) [1].

One of the plants that have the potential as an anti-inflammatory is pacing putih (*Costus speciosus* (J. Koenig) Sm.). Pacing putih is a plant of the genus *Costus* which is often found on the islands of Java and Kalimantan. The rhizome and roots of pacing putih (*C. speciosus*) contain secondary metabolites such as saponins, flavonoids, alkaloids, steroids, tannins, and phenolics. Methanol and ethanol extracts have anti-inflammatory, analgesic, and antipyretic activities [2]. In addition, infusion or decoction of the leaves as well as.

The collision of pacing putih leaves is useful for treating fever and diarrhea [3]. On Wawoni Island, Southeast Sulawesi, pacing leaves are useful for helping the postnatal

healing process and for antifertility [4]. Pacing putih stems have antioxidant activity that can reduce free radicals well [3, 5–7].

Based on the description above and chemotaxonomically, it is necessary to research anti-inflammatory activity using the in vitro protein denaturation inhibition method using UV-Visible spectrophotometer on the ethyl acetate and methanol-water fractions of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) which was determined by the value of inhibition concentration (IC50) which determines the anti-inflammatory activity of the fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.).

2 Methods

2.1 Research Procedures

Extraction

Pacing putih flowers (*Costus speciosus*) are cleaned using running water and then drained and cut into small pieces. Pacing putih flowers (*C. speciosus*) were dried at room temperature without direct sunlight and mashed into powder using a blender and weighed [8].

Pacing putih flower powder (*Costus speciosus*) obtained was extracted by maceration method using ethanol solvent for 2×24 h in dark maceration media then filtered and carried out 2 repetitions. After that, the filtrate was concentrated using a rotary evaporator to obtain a concentrated ethanolic extract of pacing putih flower (*Costus speciosus*) and weighed [9].

Phytochemical Test

Alkaloid Test

The ethyl acetate fraction and methanol-water fraction were dissolved in a suitable solvent. A total of 5 drops of H_2SO_4 and 3 drops of Dragendorff's reagent were added to each test tube. A positive test for alkaloids is indicated by the presence of orange to red-brown deposits [10].

Steroid/Triterpenoid Test

The fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was added with Lieberman-Burchard reagent (acetic anhydride with concentrated H_2SO_4) in each test tube. A positive test for steroids produces a green-blue color and triterpenoids have a purple or red color [11].

Phenolic Test

The fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was added 3 drops of $FeCl_3$ solution in each test tube. A positive test containing phenolic has a strong green, red, purple, blue, or black color [11].

Flavonoid Test

The fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was added 2 mg

of magnesium powder and then 3 drops of $\text{HCl}_{(p)}$ was added to each test tube. A positive test containing flavonoids is formed in red, green, yellow, or orange colors [12].

Quinone Test

The fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was added 3–5 drops of 5% NaOH and 5 drops of 2 N HCl in each test tube. The positive test contains quinone, and the color of the solution returns to the beginning (same as the blank color) [13].

Saponin Test

The fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was added 2 mL of hot distilled water and a few drops of $\text{HCl}_{(p)}$ in each test tube. A positive test containing saponins contained stable foam for ± 15 min [14].

Anti-Inflammatory Activity Test

Making Tris Buffer Saline (TBS)

4.35 g of NaCl crystals were dissolved with 200 mL of distilled water, then 605 mg of Tris Base was added and the volume was made up to 400 mL with distilled water. To stabilize the pH to 6.3, glacial acetic acid was used, and then the volume was filled with distilled water to 500 mL [15].

Preparation of Bovine Serum Albumin (BSA) Solution in Tris Buffer Saline (TBS)

0.2 g of BSA powder was put into a 100 mL volumetric flask then Tris Buffer Saline (TBS) solution was added to the tera mark and homogenized [15].

Preparation of Positive Control Solution

25 mg of sodium diclofenac powder was put into a 25 mL volumetric flask, then methanol was added to the tera mark, homogenized and 1000 ppm mother liquor was obtained. Next, the mother liquor was diluted with various concentrations of 50; 25; 12.5; 6.25, and 3.13 ppm [15].

Preparation of Negative Control Solution

Methanol or ethyl acetate solvent (appropriate solvent) as much as 50 L was put into a 5 mL volumetric flask and then 0.2% BSA was added in TBS to the tera mark and homogenized [15].

Preparation of Test Solution

A total of 25 mg of ethyl acetate fraction and methanol-water fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was dissolved in a suitable solvent and put into a 25 mL volumetric flask and obtained 1000 ppm mother liquor. Next, the mother liquor was diluted with various concentrations of 500; 250; 125; 62.5; 31.3 ppm [15].

Anti-inflammatory Activity Test

A total of 50 μL of positive control solution, negative control solution, and test solution, each of which was added with 0.2% BSA solution until the volume became 5 mL. Subsequently, the solution was incubated for 30 min at a temperature of ± 25 °C and 5

min at a temperature of ± 72 °C in a water bath. The next step is the solution is cooled using room temperature for 25 min and the absorbance is measured using a UV-Visible spectrophotometer with a wavelength range of 655–665 nm [15].

2.2 Data Analysis

Measurement of the percentage inhibition of protein denaturation using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Absorbance negative control solution} - \text{Absorbance test solution}}{\text{Absorbance negative control solution}} \times 100\% \quad (1)$$

The IC₅₀ value can be calculated by making a linear regression equation between concentration and % inhibition. If the % inhibition is > 20%, it can be suspected to have anti-inflammatory activity [15].

3 Results and Discussion

The maceration yield of the pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) was obtained at 52 grams with a yield of 4.84%. A total of 30 grams of ethanol extract was fractionated with methanol and water in a ratio of 8:2 then with n-hexane and ethyl acetate solvents and the yield results are shown in Table 1.

The phytochemical test was carried out qualitatively with a color test using specific reagents with phytochemical test results presented in Table 2.

Based on Table 2, the ethyl acetate fraction contains quinones and steroids, while the methanol-water fraction contains phenolics and flavonoids. In the ethyl acetate and methanol-water fractions, there were no saponins, presumably due to the small concentration of saponins so they could not be detected in the three fractions.

In the flavonoid test, flavilium salts are formed due to the reduction of flavonoid compounds in the sample with Mg²⁺ and HCl where a color change is obtained from magnesium ions interacting with phenoxy ions [16].

In the phenolic test, Fe³⁺ ions react with phenoxy ions (O⁻) by releasing H⁺ ions from their hydroxyl groups on polyphenols to form iron(III) hexaphenolate complex compounds with blackish-green color [17].

Table 1. Comparison of different photo-bioreactors

| Fraction | Mass (gram) | Yield (%) |
|----------------|-------------|-----------|
| n-Hexane | 4 | 13.33 |
| Ethyl Acetate | 10 | 33.33 |
| Methanol-water | 2 | 6.67 |

Table 2. Phytochemical Test Results of Extract and Fraction of Pacing Putih Flower (*Costus speciosus* (J. Koenig) Sm.)

| Secondary Metabolites | Ethanol Extract | Ethyl Acetate Fraction | n-Hexane Fraction | Methanol-water Fraction |
|-----------------------|-----------------|------------------------|-------------------|-------------------------|
| Alkaloids | – | – | – | – |
| Steroids | – | + | + | – |
| Triterpenoids | – | – | – | – |
| Fenolic | + | – | – | + |
| Flavonoids | + | – | – | + |
| Quinone | – | + | + | – |
| Saponin | + | – | – | – |

Note: (+) = positive contains secondary metabolites
(–) = negative contains secondary metabolites

In the saponin test, a stable foam was formed for ± 15 min which indicated that there were glycosides where saponins had hydrophobic groups (aglycones) and glucose. Glycosides can form a foam in water to produce glucose [18].

In the steroid test, an oxidation reaction occurs between sulfuric acid and the sample where the green color is obtained from the hydroxyl group (–OH) of cholesterol which reacts with Lieberman Burchard's reagent [19].

The anti-inflammatory activity test in this study was carried out in vitro to know the anti-inflammatory activity of pacing putih flowers (*Costus speciosus* (J. Koenig) Sm.) using bovine serum albumin (BSA) by protein denaturation. The principle of this method is that the solution changes to become cloudy which indicates that the protein is undergoing denaturation [20]. This method is used to limit the use of live specimens with a faster test time and a smaller number of samples [21].

Based on Table 3, the ethyl acetate fraction and the methanol-water fraction with various concentrations of 31.3; 62.5; 125; 250 and 500 mg/L obtained % inhibition >20% respectively with a concentration of 31.3 mg/L having % inhibition of 36.82% and 42.88%, respectively. The concentration of 500 mg/L was 85.39% and 89.39%, respectively.

Based on Table 4, sodium diclofenac with various concentrations of 3.13; 6.25; 12.5; 25 and 50 mg/L obtained % inhibition >20% with a concentration of 3.13 mg/L having a % inhibition of 42.53% and a concentration of 50 mg/L of 89.31%. Sodium diclofenac can bind to albumin and form a more stable protein so that the protein does not undergo denaturation. In addition, tryptophan residues can interact with bovine serum albumin (BSA) [22]. The IC₅₀ value of sodium diclofenac and fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) is presented in Fig. 1.

Based on Fig. 1, the anti-inflammatory activity of the pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) fraction was found to from strong to weak, namely the methanol-water fraction and the ethyl acetate fraction. The smaller the IC₅₀ value, the stronger the anti-inflammatory activity. Flavonoids can block the lipoxigenase and

Table 3. Test Results of Anti-inflammatory Activity Pacing Putih Flower Fraction

| Concentration Sample (mg/L) | Fraction | | | |
|-----------------------------|------------------------|----------------|-------------------------|----------------|
| | Ethyl Acetate Fraction | | Methanol-water Fraction | |
| | Average Absorbance | Inhibition (%) | Average Absorbance | Inhibition (%) |
| Negative control | 0,89 | — | 0,999 | — |
| 31,3 | 0,5632 | 36,82 | 0,5706 | 42,88 |
| 62,5 | 0,4693 | 47,27 | 0,4356 | 56,40 |
| 125 | 0,3676 | 58,70 | 0,3493 | 65,04 |
| 250 | 0,1983 | 77,72 | 0,2536 | 74,61 |
| 500 | 0,130 | 85,39 | 0,106 | 89,39 |
| IC ₅₀ | 80.1728 | | 14.9772 | |

Table 4. Test Results of Positive Control Anti-inflammatory Activity

| Concentration Sample (mg/L) | Sodium Diklofenak (Positive Control) | |
|-----------------------------|--------------------------------------|----------------|
| | Average Absorbance | Inhibition (%) |
| Negative control | 0,994 | — |
| 3,13 | 0,5713 | 42,53 |
| 6,25 | 0,4846 | 51,25 |
| 12,5 | 0,3516 | 64,63 |
| 25 | 0,2736 | 72,47 |
| 50 | 0,1063 | 89,31 |
| IC ₅₀ | 4.0947 | |

cyclooxygenase (COX) A2 pathways which function to inhibit the production of arachidonic acid to reduce the number of prostaglandins, leukotrienes, hydroperoxide acids, and prostacyclins thereby reducing inflammation [23]. In the biosynthesis of phenolic arachidonic acid which is a mediator of inflammation, namely prostaglandins, occurs due to tissue damage and free radical scavenging so that it inhibits the cyclooxygenase enzyme [24]. Steroids that can mobilize glucocorticoid receptors by increasing or decreasing the transcription process of the genes involved and inhibiting the phospholipase process so that it does not form arachidonic acid which will inhibit the cyclooxygenase enzyme in producing prostaglandins as an inflammatory medium [25, 26]. The hydroxyl group (–OH) in secondary metabolites functions as a membrane protector and inhibits the release of inflammatory mediators [21].

In the pacing putih flower fraction (*C. speciosus*) it is suspected that the anti-inflammatory agents are phenolics, flavonoids, and steroids. According to the IC₅₀ value,

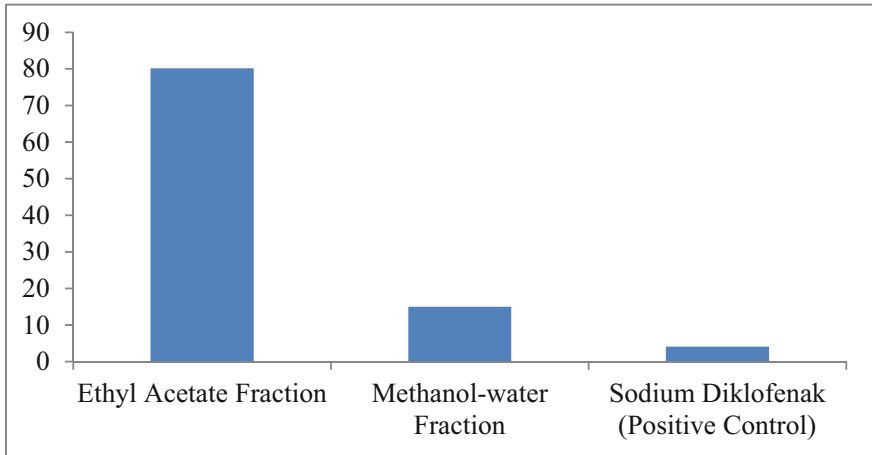


Fig. 1. Diagram of the IC₅₀ value of Sodium Diclofenac and Fraction of Pacing Putih Flower (*Costus speciosus* (J. Koenig) Sm.)

the anti-inflammatory activity can be sorted from the strongest to the weakest, namely sodium diclofenac, methanol-water fraction, and ethyl acetate fraction. The smaller the IC₅₀ value, the stronger the anti-inflammatory activity.

4 Conclusion

Based on the results of the study, it can be concluded as follows:

- The ethyl acetate fraction of pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) contains steroids and quinones. In the methanol-water fraction, there are phenolics and flavonoids.
- The value of inhibition concentration (IC₅₀) on the ethyl acetate and methanol-water fractions was 80.1728 and 14.9772 mg/L.
- In the methanol-water fraction, pacing putih flower (*Costus speciosus* (J. Koenig) Sm.) has very strong anti-inflammatory activity. In the ethyl acetate fraction, the anti-inflammatory activity was strong.

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