

Evaluation of the Anti-Inflammatory Effect of Ethanol Extract of Porang Tuber (*Amorphophallus muelleri* Blume) on White Rats (*Rattus norvegicus*)

Annisa Amaliah*, Safriani Rahman, Aulia Wati

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar 90231, Indonesia

* Corresponding Author. E-mail: amaliah021102@gmail.com

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ABSTRACT: Inflammation is a protective response to tissue injury aimed at neutralizing or eliminating invasive organisms triggered by physical trauma. One of the plants known for its anti-inflammatory properties is the porang tuber, which contains flavonoids. This study aimed to determine the anti-inflammatory effect and effective dosage of ethanol extract of porang tuber (*Amorphophallus muelleri* Blume) in male rats (*Rattus norvegicus*). An experimental design was employed, utilizing 20 white rats divided into five treatment groups: a negative control (1% NaCMC), a positive control (sodium diclofenac), and porang tuber ethanol extract at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW. Test preparations were administered one hour before the induction of 1% carrageenan (0.1 mL, intraplantar). Paw edema volume was measured plethysmographically every 60 minutes for 6 hours. Statistical analysis, performed via one-way ANOVA followed by a Bonferroni post-hoc test, revealed no significant difference between the positive control group and the porang tuber ethanol extract groups. In conclusion, the porang tuber ethanol extract exhibits anti-inflammatory effects, with a dose of 400 mg/kgBW identified as the most effective.

KEYWORDS: Anti-inflammatory; extraction; phytochemical; spectrophotometry; protective.

1. INTRODUCTION

Inflammation is the body's protective response to tissue injury, aimed at inactivating or destroying invading organisms caused by physical trauma (Fawwaz et al., 2024). The primary causes of inflammation include pressure from blunt force trauma, the entry of foreign objects, vibration, and chronic low-intensity pressure (Emelda et al., 2023). Clinically, inflammation is characterized by redness (rubor), heat (kalor), pain (dolor), and swelling (tumor) (Octavian, 2022).

In Indonesia, diseases involving inflammatory processes have a relatively high incidence rate. According to Kemenkes RI (2019), the prevalence of asthma is 2.4%, acute respiratory infections (ARI) is 9.3%, pneumonia is 4%, joint diseases are 7.3%, and cancer is 1.8%. The high incidence rates highlight the need for the development of safe and effective therapies to address inflammatory processes.

One of the conventional therapies widely used is nonsteroidal anti-inflammatory drugs (NSAIDs). These drugs aim to relieve pain, reduce inflammation, repair damaged tissue, and protect tissue from infection (Octavian, 2022). However, long-term use of NSAIDs can cause side effects, such as stomach irritation, intestinal disorders accompanied by anemia due to bleeding, and kidney damage (Idacahyati et al., 2019). Such adverse effects underscore the need for alternative therapies derived from natural ingredients with minimal side effects.

The World Health Organization (WHO) recommends the use of traditional medicine (back to nature) by utilizing the potential of natural ingredients. The use of natural ingredients, whether from plants, microbes, or other sources, generally has a lower risk of side effects compared to synthetic drugs (Latief et al., 2021). From a religious perspective, Allāh SWT has created various kinds of plants that are beneficial to humans, as explained in the Qur'an, Surah Fāṭir [35]:9;

وَاللَّهُ الَّذِي أَرْسَلَ الرِّيحَ فَتَنِّيْرُ سَحَابًا فَسُقْنَهُ إِلَى بَلَدٍ مَيِّتٍ فَأَحْيَيْنَا بِهِ الْأَرْضَ بَعْدَ مَوْتِهَا كَذَلِكَ النُّشُورُ

The translation:

"And it is Allāh Who sends the winds, so that they raise up the clouds, and We drive them to a dead land, and revive therewith the earth after its death. As such (will be) the Resurrection!" (TMNQ, 2025).

One plant with potential as an anti-inflammatory agent is porang (*Amorphophallus muelleri* Blume). This tuber crop is considered highly valuable economically and is widely cultivated by farmers as a source of additional income (Lestari et al., 2023). Empirically, porang tubers are used as antihyperglycemic, antihypertensive, antihyperlipidemic, antibacterial, and antioxidant agents. The tubers contain active compounds such as saponins, flavonoids, alkaloids, glucomannan, and calcium oxalate, while the leaves contain tannins (Hartaman et al., 2023).

Previous studies have demonstrated the various potential applications of the porang tubers. The administration of 200 mg porang flour to Wistar rats with type 2 diabetes mellitus resulted in a reduction of blood glucose levels to 176



mg/dL. Hartaman *et al.* (2023) also found that the ethanol extract of porang tubers has relatively high antioxidant activity. However, studies on the anti-inflammatory potential of ethanol extract from porang tubers in animal models are still limited. Based on the above, this study was conducted to test the anti-inflammatory effects of ethanol extract from porang tubers (*Amorphophallus muelleri* Blume) on white rats (*Rattus norvegicus*), thereby adding scientific evidence regarding the use of this plant as a natural anti-inflammatory agent.

2. EXPERIMENTAL SECTION

2.1. Population and Samples

This study was conducted at the Pharmacology Laboratory of the Faculty of Pharmacy, University Muslim Indonesia (UMI), Makassar, from August to February 2025. The study population consisted of all *Amorphophallus muelleri* Blume tubers, while the analyzed samples were obtained from tubers in Tanete Village, Bulukumpa Subdistrict, Bulukumpa Regency, South Sulawesi Province.

2.2. Working Method

This study is an experimental laboratory investigation designed to assess the anti-inflammatory effects of ethanol extract from porang tubers (*Amorphophallus muelleri* Blume) on male rats (*Rattus norvegicus*) using a plethysmometer. Twenty rats were divided into five groups: a negative control (NaCMC), a positive control (sodium diclofenac), and three treatment groups that received porang tuber ethanol extract at doses of 100, 200, and 400 mg/kgBW orally. One hour after administration of the test preparation, the animals were injected intraplantarly with 0.1 mL of 1% λ -carrageenan. The volume of paw edema was measured before induction (V_0), at 60 minutes after induction (V_1), and every hour up to 6 hours (V_1 – V_6) at 60-minute intervals using a plethysmometer.

2.3. Instruments and Materials

The instruments used in this study included beaker glasses (Pyrex®), analytical scales (Fujitsu®), hotplate stirrer (Ika®), porcelain dish (Pyrex®), oral needle (cannula), rotary evaporator (Ika®), syringe (Terumo®), water bath (Mettler®), mortar and pestle, plethysmometer, and stopwatch (Diamond®). The materials used in this study were distilled water, 96% ethanol, ethanol extract of porang tuber (*Amorphophallus muelleri* Blume), 1% λ carrageenan, and sodium diclofenac. The test animals used were healthy male Wistar rats (*Rattus norvegicus*) weighing 150 – 200 g.

2.4. Research Procedure

2.4.1. Plant determination

Plant identification was conducted at the Pharmacognosy and Phytochemistry Laboratory of the University of Muslim Indonesia using porang tubers (*Amorphophallus muelleri* Blume), including leaves, roots, stems, and fruits.

2.4.2. Sample preparation

The porang tuber (*Amorphophallus muelleri* Blume) samples were obtained from Tanete Village, Bulukumpa Subdistrict, Bulukumpa Regency, South Sulawesi Province. The tubers were peeled, washed, and cleaned of outer skin, branches, and rotten parts to prevent contamination and maintain quality. The skin was carefully removed to avoid reducing yield; then, the tubers were cut into two parts and sliced 0.5–1 mm thick, with an area of ± 15 – 20 cm² (Aryanti and Abidin, 2015). The slices are sun-dried under direct sunlight until a constant weight is achieved, followed by the determination of the post-drying moisture content.

2.4.3. Preparation of the sample solution

A total of 500 g of porang tubers (*Amorphophallus muelleri* Blume) were macerated with 3 L of 96% ethanol for three cycles, each lasting 24 hours, at room temperature with daily stirring. The maceration filtrate was evaporated using a rotary vacuum evaporator at a temperature of 50°C and a speed of 45 rpm until a thick extract was obtained. A 1% NaCMC solution was prepared by dissolving 1 g of NaCMC in 10 mL of hot distilled water, stirring until homogeneous, then adding distilled water to a total volume of 100 mL (Manengkey *et al.*, 2020). The sodium diclofenac suspension was prepared by weighing 0.97 g of powder (equivalent to 20 tablets) and then suspending it gradually in 1% NaCMC. The mixture was stirred until homogeneous, and the volume was adjusted to 100 mL in a volumetric flask (Prayitno *et al.*, 2021). The 1% carrageenan suspension was prepared by dissolving 100 mg of carrageenan in a 0.9% sodium chloride solution to a volume of 10 mL in a volumetric flask (Dermiati *et al.*, 2018). The ethanol extract suspension of porang tubers was prepared in three doses: 100, 200, and 400 mg/kgBW, with each dose dissolved in 25 mL of 1% NaCMC.

2.4.4. Selection and preparation of experimental animals

The test animals used were 24 male white rats weighing 150–200 grams, divided into five groups. The number of rats used was calculated using Federer's formula, which is:

$$(n - 1)(t - 1) \geq 15$$

Note:

n = sample per group

t = number of groups

$$\begin{aligned}(n-1)(t-1) &\geq 15 \\ (n-1)(5-1) &\geq 15 \\ (n-1)(4) &\geq 15 \\ 4n-4 &\geq 15 \\ 4n &\geq 15+4 \\ 4n &\geq 19 \\ n &\geq \frac{19}{4} \\ n &\geq 4,75 = 4 \text{ rats}\end{aligned}$$

2.4.5. Treatment of experimental animals

The prepared test animals were weighed and fasted for approximately 18 hours. The initial stage began with measuring the volume and diameter of the test animals' paws, which served as the initial volume (V_0). Next, each group of test animals was treated with either a control or test extract, as follows: group 1 received 1% NaCMC as the negative control, group 2 received sodium diclofenac as the positive control, group 3 received ethanol extract of porang tuber (EEPT) at a dose of 100 mg/kgBW, group 4 received EEPT at a dose of 200 mg/kgBW, and group 5 received EEPT at a dose of 400 mg/kgBW. After 60 minutes, the mice were induced with 1% carrageenan subplantarily, followed by measurement of paw edema volume using a plethysmometer over a 6-hour period, with measurements taken every hour.

The quantitative data of the study consisted of the AUC (Area Under the Curve) of the average edema volume curve over time and the percentage of anti-inflammatory effect. The AUC value represents the average area under the curve, which is the relationship between the average edema volume per unit of time, calculated using the formula:

$$AUC_{t_{n-1}}^{t_n} = \frac{V_{t_{n-1}} + V_{t_n}}{2} (t_n - t_{n-1})$$

Note:

$V_{t_{n-1}}$ = mean edema volume at t_{n-1}

V_{t_n} = mean edema volume at t_n

The percentage of anti-inflammatory activity (inhibition of edema volume) was calculated based on the percentage reduction in edema using the following formula:

$$\%AIA = \frac{AUC_k + AUC_p}{AUC_k} \times 100\%$$

Note:

AUC_k = mean value for the negative control

AUC_p = mean value for the treatment group

2.5. Data Analysis

The data obtained from the percentage reduction in edema volume were subsequently analyzed using a one-way ANOVA test.

3. RESULTS AND DISCUSSION

Inflammation is a protective response to tissue injury, which involves deactivating or destroying invading organisms caused by physical trauma. The main signs of inflammation include redness (rubor), heat (kalor), pain (dolor), and swelling (tumor) (Octavian, 2022). This study aimed to test the anti-inflammatory effects and determine the effective dose of ethanol extract from porang tubers (*Amorphophallus muelleri* Blume) in male rats induced with carrageenan as an anti-inflammatory agent. The anti-inflammatory testing in this study used porang tuber (*Amorphophallus muelleri* Blume) samples extracted through a maceration process with 96% ethanol. This method is fast, simple, and does not involve heating, thereby preventing damage or loss of the desired active compounds (Yasacaxena et al., 2023).

The method used was the edema formation method using 1% carrageenan. Carrageenan has several advantages, including leaving no scars, causing no tissue damage, and providing a more sensitive response to anti-inflammatory drugs (Zunnita and Auliya, 2024). Edema caused by the injection of 1% carrageenan persists for 6 hours and gradually decreases over 24 hours (Wardani, 2020). The parameter observed in this study was edema volume, measured using a plethysmometer. The principle of the plethysmometer is based on Archimedes' principle, which states that when an object is placed in a liquid, an upward force or pressure is generated (Sriarumtias et al., 2020).

The ethanol extract of porang tubers was tested for its anti-inflammatory properties in male Wistar rats (*Rattus norvegicus*). A total of 20 test animals were used, divided into five groups: Group I was given 1% Na CMC, Group II was given a suspension of sodium diclofenac 50 mg, and groups III, IV, and V were given EEPT suspension at doses of

100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW, respectively. All treatments were administered orally. The dose selection was based on previous research indicating that a dose of 400 mg/kgBW could reduce high-density lipoprotein (HDL) levels.

The hind legs of the test animals were marked as a reference when placed in the plethysmometer fluid, and their volume was measured as the initial volume (V_0). One hour after treatment, all groups were induced with 1% carrageenan at 0.1 mL subplantarily. The leg volume was then measured again as the induction volume (V_i). There are three phases of edema formation due to carrageenan induction: the first phase is the release of histamine and serotonin (up to 90 minutes), the second phase is the release of bradykinin (1.5–2.5 hours after induction), and the third phase is the release of prostaglandins (approximately 3 hours after induction), during which edema develops rapidly and reaches its maximum volume around 6 hours after induction (Dermiati et al., 2018). Edema volume measurements were taken over 6 hours at 60-minute intervals (V_t). The average edema volume data for each hour in each group was averaged and plotted in a graph, as shown in **Figure 1** below:

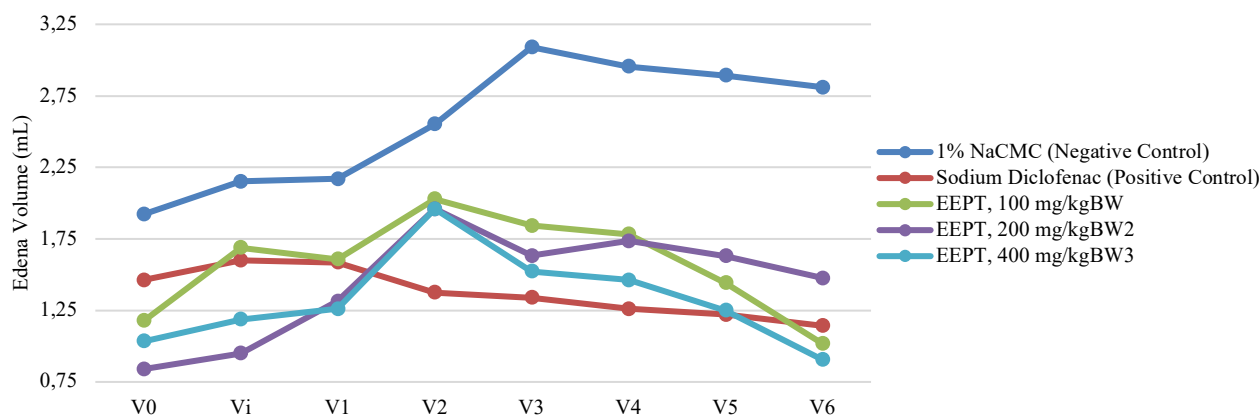


Figure 1. Graph of the average edema volume (mm^3) on the rats' feet measured using a plethysmometer

The average edema volume data for rat paws in Figure 1 shows that each group experienced a decrease in edema volume between hours 4 and 6 at 60-minute intervals. From the average edema volume results, it can be seen that in the 1% NaCMC treatment, there was an increase, but no significant decrease. In the EEPT treatment groups at doses of 100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW, there was a decrease in edema volume; however, at the 400 mg/kgBW dose, the decrease in edema volume was nearly comparable to that of the sodium diclofenac treatment group, which served as the positive control.

The measurement results presented in **Figure 1** were then processed into quantitative data in the form of AUC and the calculation of anti-inflammatory activity percentage (%AIA). The AUC value reflects the potential of the ethanol extract of porang tubers to reduce edema (inflammation). The larger the AUC value, the smaller the effect on reducing edema volume, and the smaller the AUC value, the larger the effect on reducing edema volume. The %AIA represents the percentage of a compound's ability to exhibit anti-inflammatory activity. The AUC value is inversely proportional to the %AIA value. The smaller the AUC value, the larger the %AIA value (Priamsari and Krismonikawati, 2020). The %AIA is calculated by comparing the AUC of the negative control (1% NaCMC) with the AUC values of each treatment. The AUC and %AIA values from the average edema volume curve are presented in **Table 1**.

Table 1. Average results of AUC and %AIA measurements on edema volume in each group.

Treatment	Total Area Under the Curve	Anti-Inflammatory Activity (%)
1% NaCMC (negative control)	13.98	0
Sodium diclofenac (positive control)	6.56	53
EEPT, 100 mg/kgBW	8.41	40
EEPT, 200 mg/kgBW	7.88	44
EEPT, 400 mg/kgBW	7.12	49

The average AUC values and %AIA in Table 1 indicate that the sodium diclofenac treatment group has low AUC values and high %AIA values, indicating an anti-inflammatory effect on the rat paws. Sodium diclofenac is a non-selective cyclooxygenase (COX) inhibitor with analgesic, antipyretic, and anti-inflammatory effects (Katzung et al., 2014). The mechanism of action of sodium diclofenac is to inhibit the biosynthesis of prostaglandins, inflammatory mediators, by inhibiting the cyclooxygenase enzyme (Goodman and Gilman, 2012).

In the ethanol extract of the nutmeg leaf test group, the lowest AUC value and highest %AIA were found at a dose of 400 mg/kgBW, which was close to the AUC and %AIA values in the sodium diclofenac group. Conversely, the negative control group, 1% NaCMC, showed very high AUC values and low %AIA, indicating no anti-inflammatory effect. The absence of an anti-inflammatory effect in the negative control group is attributed to the administration of only

a 1% NaCMC suspension, which serves as a mere suspending agent without any pharmacological effects or influence on edema reduction (Prayitno *et al.*, 2021).

The edema volume (AUC) values obtained were further analyzed using one-way ANOVA statistics. Normality testing was performed using the Shapiro-Wilk method, which showed that the data were normally distributed ($p > 0.05$). After normality testing, homogeneity testing was performed using the Levene method to determine whether the data on the percentage of edema reduction in rat paws were homogeneous. The homogeneity test results showed that the data were homogeneous ($p > 0.05$). Thus, the requirements for the one-way ANOVA test were met.

The one-way ANOVA test results revealed significant differences in edema volume reduction among treatment groups ($p < 0.05$). Since the requirements were met, a Bonferroni post-hoc test was performed to determine the differences between groups. The results of the Bonferroni test showed that the 1% NaCMC group differed significantly from the sodium diclofenac group and all test extract doses (100 mg/kgBW, 200 mg/kgBW, and 400 mg/kgBW) ($p < 0.05$). This result indicates that NaCMC does not affect reducing edema volume in rat paws.

Meanwhile, the sodium diclofenac group did not differ significantly from any of the test extract groups ($p > 0.05$), indicating a similar effect in reducing edema volume. Furthermore, the 400 mg/kgBW extract dose did not differ significantly from the 100 mg/kgBW and 200 mg/kgBW doses ($p > 0.05$), indicating that all three doses had the same effect in reducing paw edema volume in rats.

Based on the research results, the ethanol extract of the porang tuber (*Amorphophallus muelleri* Blume) demonstrated anti-inflammatory effects at an effective dose of 400 mg/kgBW. This effect is believed to originate from the presence of secondary metabolites such as flavonoids, alkaloids, and saponins. Flavonoids act as anti-inflammatory compounds by reducing the production of prostaglandins and leukotrienes through the inhibition of COX and lipoxygenase enzymes in the inflammatory process (Putri *et al.*, 2024). Alkaloids work by inhibiting the release of histamine by mast cells (Wasiaturrahmah and Amalia, 2023). The mechanism of saponins as anti-inflammatory agents involves inhibiting the formation of exudate and increasing vascular permeability (Rahajeng and Permana, 2020).

4. CONCLUSION

Based on the research results, it can be concluded that the ethanol extract of porang tubers (*Amorphophallus muelleri* Blume) has anti-inflammatory effects on male rats (*Rattus norvegicus*). A dose of 400 mg/kgBW is the most effective dose in reducing edema volume in test animals.

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Ethical Approval: Not applicable

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