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Ethanolic Extract of Fingerroot (*Boesenbergia pandurata* Roxb.) Rhizomes exhibits Analgesic and Anti-Inflammatory Activities in Swiss Webster Mice

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Abstract
Pain is a sensory response that indicates tissue damage caused by mechanical, chemical, or physical stimuli. This study aimed to evaluate the analgesic and anti-inflammatory activities of the ethanol extract of fingerroot rhizomes (EEFR) in mice. The extract was prepared by maceration using ethanol and tested in mice divided into five groups: three treatment groups receiving EEFR at doses of 100, 200, and 400 mg/kgBW, a positive control group, and a negative control group. Analgesic activity was assessed by the number of writhing responses induced by 1% acetic acid, while anti-inflammatory activity was evaluated based on paw edema induced by 1% carrageenan. The results showed that EEFR at a dose of 400 mg/kgBW produced the most significant analgesic and anti-inflammatory effects, with pain protection and inflammation inhibition percentages of 69.81% and 58.97%, respectively. These findings indicate that EEFR has potential as a natural analgesic and anti-inflammatory agent.

Keywords: analgesic, anti-inflammatory, extract, fingerroot, mice

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Ekstrak Etanol Rimpang Temu Kunci (*Boesenbergia pandurata* Roxb.) Menunjukkan Aktivitas Analgesik dan Antiinflamasi pada Mencit Swiss Webster

Abstrak
Nyeri merupakan respons sensorik yang menandakan adanya kerusakan jaringan akibat rangsangan mekanis, kimia, atau fisik. Penelitian ini bertujuan untuk mengevaluasi aktivitas analgesik dan antiinflamasi ekstrak etanol rimpang temu kunci (EEFR) pada mencit. Rimpang temu kunci diekstraksi dengan metode maserasi menggunakan etanol, kemudian diuji pada mencit yang dibagi menjadi lima kelompok, yaitu tiga kelompok perlakuan: 100, 200, dan 400 mg/kgBB, kontrol positif, dan kontrol negatif. Aktivitas analgesik dinilai melalui jumlah geliat setelah induksi asam asetat 1%, sedangkan aktivitas antiinflamasi dievaluasi berdasarkan edema kaki mencit yang diinduksi karagenan 1%. Hasil menunjukkan bahwa EEFR dosis 400 mg/kgBB memberikan efek analgesik dan antiinflamasi terbaik, dengan persentase proteksi nyeri sebesar 69,81% dan inhibisi inflamasi sebesar 58,97%. Temuan ini menunjukkan bahwa EEFR berpotensi sebagai agen analgesik dan antiinflamasi alami.

Kata Kunci: analgesik, anti-inflamasi, ekstrak, temu kunci, mencit

1. Introduction

Pain is a sensory and emotional experience that arises in response to actual or potential tissue damage. Such damage may result from various stimuli, including mechanical, chemical, and physical factors (e.g., heat and electricity), which can trigger painful sensations. Pain serves as a protective warning signal, alerting the body to potential harm or abnormal conditions.[1]

Pain often arises as a prominent manifestation of inflammatory processes in the body. Inflammation is an immune response activated by harmful stimuli, such as invading microorganisms, toxic substances, or damaged cells. Through this response, the immune system works to eliminate these injurious factors while simultaneously promoting the repair and regeneration of affected tissues.[2]

One common approach to reducing inflammation and pain is the use of nonsteroidal anti-inflammatory drugs (NSAIDs), which are widely prescribed to alleviate fever, inflammation, and pain. However, long-term or frequent use of NSAIDs may lead to various adverse effects, particularly gastrointestinal disturbances. Consequently, increasing attention is being directed toward the use of natural products as alternative therapeutic options for managing these conditions.[3]

The World Health Organization (WHO) recommends using medicinal plants to support public health, prevent disease, and treat various illnesses. Increasing public awareness of health has also contributed to the widespread use of herbal medicines, which Indonesians have long used. This preference is driven by their proven therapeutic efficacy, lower cost, and relatively fewer side effects. One medicinal plant with strong potential for cultivation and further development in Indonesia is temu kunci (*Boesenbergia pandurata* Roxb.).

Temu kunci, or Fingerroot, is a traditional medicinal plant commonly used by society as a pain reliever, expectorant, worm medicine, and appetite stimulant.[4] Fingerroot contains several secondary metabolites which have various biological and pharmacological activities, including free radical scavenging, antitumor, antiangiogenic, anti-inflammatory, anti-obesity, and anti-oxidant.[5] Based on an *in silico* study conducted by Yuniarto et al. (2022)[6], QSAR analysis results showed that the active compounds from fingerroot rhizome have several biological activities, including analgesic and anti-inflammatory properties. Therefore, in this work, male Swiss-Webster mice were used to test the analgesic and anti-inflammatory properties of ethanol extracts of fingerroot rhizomes.

2. Materials and Method

2.1. Tools

The tools used in this research were mice cages, digital plethysmometers, rotary evaporator, and laboratory glassware.

2.2. Materials

Fingerroot was obtained from Balai Penelitian Tanaman Rempah dan Obat (BALITTRO) Bogor. All materials used in this study were categorized as analytical grade.

2.3. Methods

2.3.1. Plant Determination

The plant identification was conducted at the Biology Learning Laboratory, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan, Yogyakarta.

2.3.2. Extract Preparation

Fingerroot rhizomes were extracted using the maceration method. A total of 1 kg of fingerroot rhizomes was then macerated using 96% ethanol for three 24-hour periods. The filtrate was filtered and condensed to produce the extract at 40°C using a rotary evaporator. Furthermore, the yield following extraction was calculated. The following formula was used to determine the yield:

$$\%\ Yield = \frac{extract\ weight\ obtained\ (g)}{dry\ powder\ weight\ before\ extraction\ (g)}\ x\ 100\%$$

2.3.3. Phytochemical Screening

Phytochemical screening is conducted to detect secondary metabolites, including alkaloids, flavonoids, saponins, tannins, quinones, steroids, and triterpenoids.

2.3.4. Experimental Animal

A total of 25 healthy male Swiss Webster mice (aged 2–3 months and weighing 20–30 grams) were used as test animals. Before treatment, the mice were acclimatized for seven days at room temperature to ensure their well-being and adaptation to the laboratory environment. During the acclimatization period, the mice had *ad libitum* access to drinking water and a standard diet. The 25 male mice were then randomly assigned to five treatment groups: three groups receiving ethanol extracts of fingerroot rhizome, a negative control group (0.5% Na CMC), and a positive control group (mefenamic acid 65 mg/kgBW, Na diclofenac 6.5 mg/kgBW). (*Boesenbergia pandurata*

Roxb.) – EEFR with doses of 100, 200, and 400 mg/kgBW. The doses of 100, 200, and 400 mg/kgBW were selected based on a previous study by Yuniarto et al. (2024),[7] which investigated the pharmacological activity of fingerroot rhizome extract in an anti-obesity model. Although the therapeutic target in the present study differs, the dose range was adopted as a reference because it was safe and biologically active in vivo. This approach was intended to ensure that the selected doses were within an effective and tolerable range for mice. Therefore, the same dose was used in this study to evaluate the extract's analgesic and anti-inflammatory activities. Each group comprised 5 mice. This study was conducted in the Pharmacology Laboratory at Muhammadiyah A.R. Fachruddin University, Tangerang, Indonesia. This research has received approval from the Research Ethics Committee of Ahmad Dahlan University, Yogyakarta, under the number REC-UAD/02/02/01-2025/002.

2.3.5. Analgesic Activity Test

The chemical induction method (Sigmund's method) was employed to assess the analgesic activity. The chemical administered was acetic acid. In this study, acetic acid was administered as a pain inducer, characterized by a writhing effect. After 15 minutes of extract administration, an intraperitoneal injection of 1% acetic acid was given at 0.2 ml per 20 grams of body weight.[8] The cumulative number of mice writhing was counted every 15 minutes for 60 minutes. Writhing was indicated by the mouse's legs and arms being pulled forward, with the abdomen touching the floor. The magnitude of the resistance to writhing was calculated using the formula:

$$\% \text{ Protection} = 100 - [\frac{a}{b}] \times 100\%$$

Description:
a = Cumulative number of writhing of test animals; b = Cumulative number of negative control stretches

The effectiveness of an analgesic effect can be calculated using the formula:

$$\% \text{ Effectiveness} = \frac{\%Protection\ of\ test\ group}{\%\ Protection\ of\ positive\ control} \text{ x } 100\%$$

2.3.6. Anti-Inflammation Activity Test

Inflammation induction method using subplantar carrageenan. A digital plethysmometer was used to measure edema on the mice's foot soles, providing precise, accurate measurements of paw swelling to assess the degree of inflammation. The hind paws of the mice to be induced were marked, and their volume was evaluated before treatment (V0). Next, each group received the extract. After 30 minutes, each treatment group was subplantarly injected with 0.1 ml of 1% carrageenan on the left hind paw of each mouse. The volume of edema formed was measured at 1, 2, 3, and 4 hours after carrageenan induction (Vt) using a digital plethysmometer. The percentage of edema and inhibition of edema formation can be calculated using the formula[9]:

$$\text{Percentage of edema} = \frac{Vt - Vo}{Vo} \times 100\%$$

Description:
Vt: Volume of hind paw at time t
V0: Volume of the initial hind paw

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

Description:
a: percentage of edema in the negative control
b: percentage of edema in treatment group

The greater the percentage of edema inhibition, the stronger the test material's anti-inflammatory effect.

2.3.7. Statistical Analysis

SPSS version 25 was then used to statistically analyze the data collected for this investigation. One-way ANOVA was used to examine the findings of the tests for analgesic and anti-inflammatory efficacy, followed by a post hoc LSD test. A $p<0.05$ was considered to indicate a statistically significant difference.[10]

3. Result

3.1. Plant Determination

The plant determination results indicate that the plant studied is a fingerroot, Boesenbergia Pandurata, of the *Zingiberaceae* family (No 491/Lab.Bio/B/IX/2024).

Table 1. Phytochemical screening of the extract.

Secondary Metabolites	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannin	-
Steroid/triterpenoids	-

Description:
(+): detected; (-): undetected

Table 2. Analgesic effects of EEFR determined by the writhing test method.

Groups	Reaction time (min)				Cumulative of Writhes
	15	30	45	60	
Negative Control (-)	34.80 ± 2.68	27.40 ± 6.02	22.40 ± 6.80	17.40 ± 1.52[b]	102
Positive Control (+)	5.00 ± 0.71	3.80 ± 0.45	1.80 ± 0.84	0.80 ± 0.84[a]	11,4
EEFR 100 mg/kgBW	24.20 ± 2.59	21.60 ± 1.34	17.20 ± 1.79	11.80 ± 0.84[a,b]	74.8
EEFR 200 mg/kgBW	18.20 ±1.30	16.40 ± 1.67	12.00 ± 2.55	8.80 ± 1.30[a,b]	55.4
EEFR 400 mg/kgBW	10.20 ± 0.84	9.60 ± 1.14	6.80 ± 1.64	4.20 ± 1.30[a,b]	30.8

a: p<0.05 compared with the negative group.
b: p<0.05 compared with the positive group.

3.2. Yield of Extract Percentage and Phytochemical Screening of Extract

The yield of the ethanol extract of fingerroot rhizome was 9.15%. The phytochemical screening results of the extract revealed the existence of saponins and flavonoids (Table 1). Nevertheless, no quinones, alkaloids, tannins, or steroids/triterpenoids were found.

3.3. Analgesic Activity Test

The results of the analgesic activity test using the writhing method in this study are shown in Table 2, and the percentage of effectiveness is shown in Table 3.

The analgesic effect was tested using the Writhing method. This method was chosen because it is simple and allows for rapid evaluation of analgesic testing. The writhing in the test animals is caused by the release of prostaglandins, which then trigger the sensation of pain. The use of acetic acid was chosen because it can cause significant and observable local pain in the abdominal cavity. Based on Table 2, the EEFR and positive control groups revealed a difference in standard to the negative control group, in terms of the average number of writhing. This indicates an analgesic effect of the EEFR and mefenamic acid administered. The total number of writhings exhibited by the mice indicates the intensity of pain induced by acetic acid. The greater the analgesic effect of the EEFR, the lower the number of writhes exhibited by the mice. These results indicate that the average number of writhing was highest in the negative control group. This is because the negative control group did not use any substances that could reduce pain. In the negative control group, only Na CMC was used, serving as a vehicle with no effect. Furthermore, there was a significant reduction in total writhing in all three EEFR dose groups compared to the negative control group.

3.4. Anti-Inflammation Activity Test

The percentage inhibition of inflammation results is shown in Table 4, and the time course of the EEFR's anti-inflammatory effect is shown in Figure 1.

The anti-inflammatory test conducted in this study used an artificial edema method, induced by injecting 0.1 mL of 1% carrageenan into the left paw of mice subplantarly. Carrageenan induced acute inflammation without causing tissue damage.11 Measurement of edema volume using a digital plethysmometer. A significant reduction in inflammatory volume was observed across all three EEFR dose groups compared with the negative control group. This suggests that EEFR administration can inhibit the carrageenan-induced inflammatory process, as indicated by a reduction in paw volume in mice. Based on the calculation results in Table 4, the EEFR group with the highest percentage of inhibition was 400 mg/kg b.w. dose, which was 58.97%. Based on the edema inhibition data, the 400 mg/kg body weight dose in the dose test group showed the best activity in suppressing inflammation. Meanwhile, the positive control group, namely Diclofenac Sodium at 6.5 mg/kg b.w., showed an inhibition percentage of 80.30%.

4. Discussion

EEFR administration can reduce acetic acid-induced pain, as evidenced by fewer writhing episodes in mice. The reduction in writhing response was further quantified by calculating the percentage protection

Table 3. Percentage of protection and effectiveness of analgesics.

Groups	Protection (%)	Effectiveness of Analgesic (%)
Positive Control (+)	88.83%	100%
EEFR 100 mg/kgBW	26.67%	30.02%
EEFR 200 mg/kgBW	45.69%	51.43%
EEFR 400 mg/kgBW	69.81%	78.58%

Table 4. Percentage inhibition of inflammation induced by carrageenan.

Groups	Paw volume reduction (%)				Mean of Paw volume reduction (%)
	1 Hour	2 Hour	3 Hour	4 Hour	
Positive Control (+)	68.06 ± 26.66	74.41 ± 19.01	84.82 ± 9.67	93.88 ± 6.78	80.30 ± 9.07
EEFR 100 mg/kgBW	33.99 ± 26.97	15.41 ± 18.16	16.93 ± 16.57	47.53 ± 22.37[a]	28.46 ±4.66
EEFR 200 mg/kgBW	41.33 ± 19.20	32.62 ± 20.95	22.34 ± 22.82	56.31 ± 21,61[a]	38.15 ± 1.51
EEFR 400 mg/kgBW	52.44 ± 23.93	56.52 ± 34.24	34.38 ± 26.70	92.53 ± 6.88[b]	58.97 ± 11.56

a: p<0.05 compared with the positive group.
b: p>0.05 compared with the positive group.

and percentage analgesic effectiveness. Percentage protection reflects the extent to which the test substance reduces the acetic acid-induced writhing response in mice, indicating its analgesic potential.[12,13] The higher the dose given to the test animals, the higher the percentage of effectiveness. Therefore, the EEFR test dose group showed a positive analgesic effect, as evidenced by a decrease in overall writhing.

The analgesic activity of EEFR is likely influenced by secondary metabolites, namely flavonoids and saponins. Flavonoids and saponins can relieve pain and inhibit the enzyme cyclooxygenase, thereby reducing prostaglandin formation.[14] A test substance that can reduce writhing by 50% or more may be inferred to have an analgesic effect. Based on the research results, EEFR with a test dose of 400 mg/kgBW had an effect that was close to the effectiveness of it can be concluded that this dosage best inhibits the quantity of writhing induced by pain stimulation because it is the positive control. ($p < 0.05$).

The anti-inflammatory properties of EEFR are likely due to secondary metabolites such as saponins and flavonoids. Flavonoids exert their effects by directly inhibiting the activity of cyclooxygenase (COX) and lipoxygenase enzymes, which play key roles in the inflammatory process by mediating the synthesis of pro-inflammatory mediators like prostaglandins and leukotrienes. This inhibition helps reduce inflammation and associated symptoms, which inhibits prostaglandin and leukotriene biosynthesis. Saponins, on the other hand, play a role in inhibiting exudate formation and vascular permeability.[14] As additional information, several bioactive metabolites in fingerroot rhizomes, particularly flavonoids such as pinostrobin, have been investigated for their anti-inflammatory potential.

Recent molecular docking studies demonstrate that pinostrobin derivatives exhibit high binding affinity for the COX-2 receptor, suggesting a mechanistic basis for inhibiting pro-inflammatory prostaglandin synthesis via the COX pathway.[15] Fingerroot extracts containing panduratin A and pinostrobin are safe in sub-chronic administration, supporting their suitability for in vivo pharmacological evaluation.[16] In an animal model of SARS-CoV-2 infection, fingerroot extract significantly reduced inflammatory mediators such as PGE_2 and IL-6, which are influenced by COX-mediated pathways.[17] These findings collectively support the notion that secondary metabolites in fingerroot may exert analgesic and anti-inflammatory effects through modulation of COX-related inflammatory mechanisms, consistent with the results observed in the current study.

These findings demonstrate that our work aligns with previous *in silico* studies that have shown fingerroot to have promising potential as an anti-inflammatory agent.[6] Our research certainly has limitations. We hope that EEFR can be further developed as an anti-inflammatory drug. However, preclinical data must be developed gradually to ensure its safety, and formula development must continue to ensure that the natural active ingredient is more stable, safe, effective, and acceptable to users.[18-21]

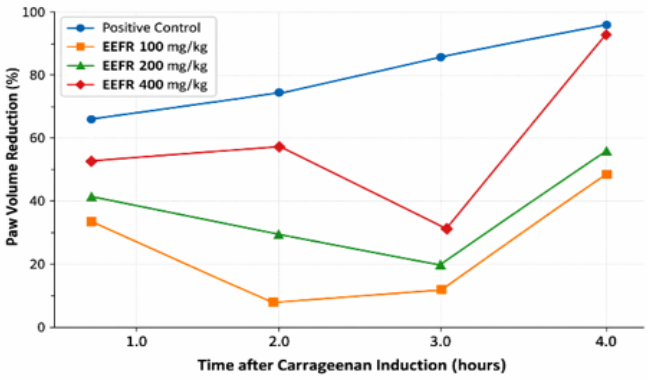

Figure 1. Time-course of the anti-inflammatory effect of EEFR.

5. Conclusion

It can be concluded that EEFR has analgesic and anti-inflammatory activity. The most effective dose for analgesic and anti-inflammatory effects in Swiss Webster mice was 400 mg/kgBW.

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Conflict of Interest

The authors declare no conflicts of interest.

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