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RESEARCH ARTICLE

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Evaluation of analgesic activity of ethanol and ethyl acetate extracts from basil leaves (*Ocimum x africanum* L.) in acetic acid-induced rat models

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Abstract: Analgesics are medications that alleviate pain without impairing consciousness, with certain plants, such as basil (*Ocimum x africanum* L.), showing potential as natural pain relievers. This research investigated the analgesic effects of ethanol and ethyl acetate extracts from basil leaves on acetic acid-induced pain in rats. The study utilized percolation extraction methods with 96% ethanol and ethyl acetate solvents. Twenty-eight male white rats were divided into seven groups: one normal control group without treatment or induction, one negative control group receiving 0.5% CMC-Na, two groups treated with ethanol extracts of basil leaves at doses of 400 mg/kg BW and 800 mg/kg BW, two groups treated with ethyl acetate extracts at the same doses, and one positive control group administered 50 mg/kg BW diclofenac sodium. Thirty minutes post-treatment, 1% acetic acid was injected intraperitoneally, and writhing responses were observed over 60 minutes. The data on percentage protection from writhing were analyzed using One-way ANOVA followed by the LSD test. The findings revealed that both the ethanol and ethyl acetate extracts at an 800 mg/kg BW dosage closely matched the analgesic effectiveness of the diclofenac sodium group.

Keywords: basil leaves, analgesic, diclofenac sodium, male white rat

Introduction

Pain conditions continue to burden society significantly, causing discomfort and painful sensations. Pain is associated with avoidance reflexes and alterations in autonomic output. It is categorized into somatic and visceral pain. Somatic pain arises in the skin, muscles, joints, bones, or connective tissues. In contrast, visceral pain originates from smooth muscle spasms, tension in abdominal organs, insufficient blood flow, and inflammation-related diseases [1].

Efforts to develop analgesics have been ongoing. Analgesics are classified into opioid and non-opioid categories. Opioid analgesics can lead to dependence and tolerance with continuous use, whereas non-opioid or peripheral analgesics do not [2] [3].

Certain diseases cause persistent pain, and chronic use of analgesics may adversely affect the body. Hence, there is a growing interest in developing alternative approaches, such as using ingredients with analgesic properties that can be consumed as food to relieve mild pain. Today's global lifestyle trend leans towards natural remedies, particularly in medicine. In Indonesia, many plants are used in traditional medicine, including those containing secondary metabolite compounds with analgesic properties, such as *Ohwia caudata* [4], the genus Trillium [5], *Rhododendron micranthum* [6], and basil (*Ocimum x africanum* L) [7].

Basil leaves contain flavonoids, phenols, saponins, and essential oils [8]. In addition, basil can function as an antibacterial [9] and hepatoprotector [10]. Flavonoids, in particular, inhibit cyclooxygenase and lipoxygenase enzymes, which are crucial in releasing pain mediators. Thus, inhibiting these enzymes can suppress pain stimuli. Given this background, further research is warranted to compare the analgesic effects of ethanol and ethyl acetate extracts from basil leaves on male white rats (*Rattus novergicus*).

Method

Sample preparation

Basil leaves were sourced from Bandar Lampung, Lampung, Indonesia. Verification were conducted at the Biology Laboratory of the University of Lampung to confirm the identity of the plants utilized in the study. The leaves were cleansed with running water and then oven-dried for 2-3 hours at 50°C.

Subsequently, the dried leaves were ground into powder for extraction.

Preparation of extract

For each solvent, 350 grams of basil leaf powder was added to 4 liters of either ethanol or ethyl acetate in a percolator, ensuring the simplisia was fully submerged. The setup was left to stand for 3 hours, allowing percolation at a rate of 1 mL per minute. The solvent was added repeatedly until the percolate ran clear. The extract was then filtered and evaporated at 40°C using a rotary evaporator, yielding thick ethanol and ethyl acetate extracts.

Determination of flavonoid content

Maximum wavelength determination: Initially, quercetin was dissolved in a solvent to prepare a 60 ppm stock solution. Then, 1 mL of this solution was mixed with 1 mL of 2% $AlCl_3$ and 1 mL of 120 mM potassium acetate in a test tube. Absorbance readings were taken using a UV-Vis spectrophotometer at the previously established maximum wavelength.

Standard curve preparation: Quercetin (25 mg) was dissolved in 25 mL of 96% ethanol to create a stock solution. This solution was then diluted to prepare standard solutions of 10, 20, 30, 40, and 50 ppm. To each standard solution, 1 mL of 120 mM potassium acetate and 1 mL of 2% $AlCl_3$ were added. After incubation at room temperature for a stable time, absorbance was measured at the maximum wavelength.

Preparation of extract test solution

Ethanol extract: A 10 mg sample of basil leaf extract was dissolved in 5 mL of 96% ethanol. The solution was then transferred to a volumetric flask and diluted to the mark to achieve a 1000 ppm concentration. Further dilutions were made to obtain 100 ppm. Then, 0.2 mL of this solution was mixed with 1.5 mL of 96% ethanol, 0.1 mL of 10% aluminum chloride, 1 mL of potassium acetate, and 2.8 mL of distilled water. The mixture was homogenized and incubated before measuring absorbance at 430 nm.

Ethyl acetate extract: The procedure mirrored that of the ethanol extract, adjusting concentrations as necessary for the determination of total flavonoid content, followed by incubation and absorbance measurement at 430 nm.

Analgesic activity test

The study involved 28 male white rats aged 2-3 months, divided into seven groups, each comprising four rats. Before testing, the rats underwent an adaptation period and were fasted for approximately 18 hours. This research adhered to strict ethical guidelines to ensure the humane treatment of all animal subjects. Measures included avoiding harm to the animals, ensuring they received adequate nutrition throughout the study period, and maintaining cleanliness by regularly cleaning the cages. The experimental groups were designated as follows:

1. Normal control: No induction or treatment was administered.
2. Negative control: Administered sodium carboxymethyl cellulose 0.5%.
3. Treatment 1: Received ethanol extract at 400 mg/kg body weight (BW).
4. Treatment 2: Received ethanol extract at 800 mg/kg BW.
5. Treatment 3: Received ethyl acetate extract at 400 mg/kg BW.
6. Treatment 4: Received ethyl acetate extract at 800 mg/kg BW.
7. Positive control: Administered diclofenac sodium at 50 mg/kg BW.

Following a single oral dose and a 30-minute absorption period, pain was induced using 1% acetic acid. The characteristic writhing response in the rats was then observed and recorded every 5 minutes for a duration of 60 minutes [11].

Data analysis

The analgesic effect was evaluated based on the reduction in writhes, with % protection calculated as 100 - (test/control x 100%). Data were analyzed using One-way ANOVA to assess the analgesic efficacy of the extracts.

Results

Basil leaf extraction

Extraction was performed using two solvents, ethanol and ethyl acetate, chosen for their low toxicity and respective polarities, ethanol being polar and ethyl acetate semipolar. A total of 350 grams of basil leaves were subjected to extraction with 4 liters of 96%

Table 1. Extract yield of basil leaves

Solvent	Powder weight (gram)	Extract weight (gram)	Yield (%)
Ethanol 96%	350	45.17	10.26
Ethyl acetate	350	35.48	10.13

Table 2. Results of determination of flavonoid levels

Sample	Replication	Absorbance	Concentration (%)	Average (%)
Ethanol extract of basil leaves	1	0.208	3.49	3.59
	2	0.235	3.96	
	3	0.197	3.31	
Ethyl acetate extract of basil leaves	1	0.159	3.02	2.80
	2	0.178	3.02	
	3	0.143	2.37	

ethanol, yielding 45.17 grams of extract, corresponding to a yield of 10.26%. Similarly, extraction with ethyl acetate resulted in 35.48 grams of extract, equating to a yield of 10.13% (Table 1).

Determination of flavonoid levels

The flavonoid content within the extracts was determined using a standard quercetin solution. $AlCl_3$ and potassium acetate facilitated the identification of the maximum absorption wavelength via UV-Vis spectrophotometry, which was determined to be 430 nm. The analysis yielded a linear regression equation ($y = 0.022x + 0.0263$) and a correlation coefficient (r) 0.9858. The flavonoid content was calculated using this equation as 3.59% in the ethanol extract and 2.80% in the ethyl acetate extract (Table 2).

Analgesic activity test

The analgesic efficacy was assessed based on the extracts' ability to mitigate writhing responses in rats induced with acetic acid. The measure of efficacy, termed "percent protection," is presented in Figure 1. The analysis revealed that the positive control, diclofenac sodium, afforded the highest percent protection at 97.53%. Among the treatments, basil leaf extracts administered at 800 mg/kg body weight for both ethanol and ethyl acetate extracts demonstrated the highest level of protection in rats.

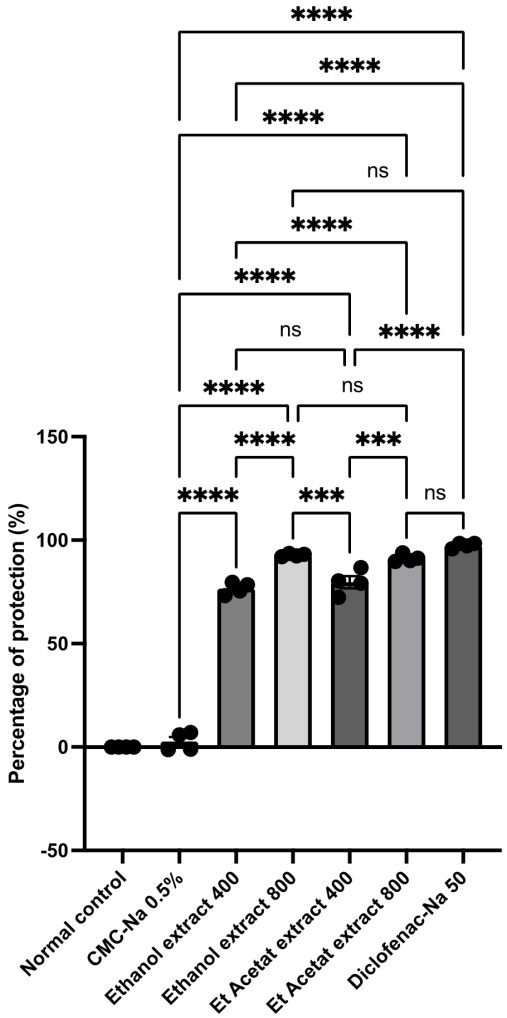

Figure 1. Results of the percentage of protection against acetic acid induction

Discussion

A substance is considered to possess analgesic properties if it significantly reduces the writhing response in rats by more than 50% compared to the negative control group, as per established criteria [12]. The study into the analgesic effects of 96% ethanol and ethyl acetate extracts of basil leaves demonstrated that all tested groups exhibited significant analgesic activity, reducing pain stimulation by over 50%.

The analgesic efficacy within the test groups was assessed by comparing their percentage of protection to that of the diclofenac sodium group. Notably, the treatment group, specifically the extract administered at a dose of 800 mg/kg body weight, approached the analgesic efficacy of diclofenac sodium, with percentages of 95.1608% for the ethanol extract and 93.6127% for the ethyl acetate extract.

Statistical analysis, including the Shapiro-Wilk test for data normality and tests for homogeneity, indicated that the data regarding the percentage of writhing inhibition were normally distributed and homogeneous (significance $p>0.05$). This allowed for further analysis using One-Way ANOVA. Subsequent testing with the Least Significant Difference (LSD) method revealed that treatments with 200 mg/kg body weight of the extract demonstrated comparable percentages of writhing inhibition, with no significant difference observed between the ethanol and ethyl acetate treatments.

Moreover, LSD test outcomes for the 800 mg/kg body weight dose of basil leaf extract showed a significance value of 0.504 ($p>0.05$), indicating that at this dosage, both the ethanol and ethyl acetate extracts exhibit analgesic effectiveness comparable to that of diclofenac sodium.

Conclusion

Analgesic activity assays of ethanol and ethyl acetate extracts of basil leaves at a dosage of 800 mg/kg body weight yielded analgesic effects of 95.16% and 93.61%, respectively. These findings underscore the significant potential of basil leaf extracts as effective analgesic agents.

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Declaration of interest

None.

Author contributions

US, N conceptualized the study design; US investigated the data; US, N, MPA wrote the original draft. All authors reviewed, edited, and read the final version of this manuscript.

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