

# Ethanol and Methanol Extract of Analgesic Activities of Ganitri Leaves (*Elaeocarpus ganitrus* Roxb) for in Vivo

Naelaz Zukhruf Wakhidatul Kiromah\*; Chondroso Miyarso; Yuyu Krisdiyanti

Departement of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Muhammadiyah Gombong, Jl. Yos Sudarso No. 461, Gombong, Kebumen, Jawa Tengah - 5441.

## Abstract

Pain is a feeling of discomfort caused by intense or destructive stimuli, which can affect your daily routine if left untreated. Pain can be treated with an analgesic. One of the plants that are considered to have analgesic effects is the leaves of the ganitri. This research aims to determine ethanol and methanol extracts' impact on decreasing analgesic activity and percent protection. The study began with collecting and processing the leaves of the ganitri into the ethanol and methanol extracts using the maceration method. The research was continued with in vivo analgesic activity testing of 24 mice induced by pain using 1% acetic acid. The induced mice were divided into eight treatment groups, where the mice in the first group served as a negative control group. In that group, they were given CMC at a dose of 0.5%. The second group was positive control, given mefenamic acid at a dose of 500 mg/kg BW. In contrast, the third until eighth groups were given ethanol and methanolic extracts of the ganitri leaves with consecutive doses of 100 mg/kg BW, 200 mg/kg BW dan 400 mg/kg BW. Parameters measuring the effectiveness of the extracts used in this study included the amount of stretching, the percentage of analgesic power, and analgesic effectiveness. The results showed that the ethanolic and methanolic extract had the highest percentage of analgesic power at 400 mg/Kg BW amounted to 91.3% and 88.3%. Furthermore, based on the statistical analysis results using ANOVA, it was found that the ethanol and methanolic extracts of the leaves of the ganitri dosage of 400 mg/Kg BW had analgesic activity close to 500 mg/kg BW of mefenamic acid.

**Keywords:** acetic acid; analgetic; ganitri leaves; mice; mefenamic acid

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## INTRODUCTION

Indonesia is rich in natural resources that can potentially become medicine precursors. More than 20.000 types of medicinal plants are scattered all around

Indonesia. About 1.000 plants have been identified, and only 300 have been used in traditional medicine.<sup>1</sup>

Pain is one of the general problems that occur in society. It is a primary factor that

\* Corresponding author, e-mail: [naela.zukhruf18@stikesmuhgombong.ac.id](mailto:naela.zukhruf18@stikesmuhgombong.ac.id)

people come to see a doctor since pain disturbs sufferers' social function and life quality. Pain is defined as sensory and emotional experience connected with tissue dysfunction (Merskey and Bogduk, 1994; Salman, Saputri and Mustika, 2021). Compounds in therapeutic dosage slow down or suppress pain without having general anesthesia, called analgesic. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain, analgesic, and inflammatory conditions.<sup>2</sup> However, they have common side effects like ulcers, bleeding, and renal disorders.<sup>3</sup> In recent years, study on herbal plants has been conducted concerning efficiency and side effects of chemical medicine.<sup>4</sup> On the other hand, natural resources have advantages such as practical efficacy, good tolerance, fewer side effects, and allergy.<sup>5</sup>

One of the traditional plants that can be used as medicine is ganitri (*Elaeocarpus ganitrus* Roxb). Ganitri contains alkaloids, flavonoids, polyphenol, tannins, saponins, and fatty acid<sup>6</sup>. It is effectively used in the treatment of inflammation and analgesic.<sup>7</sup> Research by Nain, Garg, and Dhahiya (2012) showed an analgesic of ganitri leaf aqueous extract at doses 100 mg/Kg BW. Based on the above findings, analgesic activities were evaluated in the present study. The purpose of this research is to see if there is an analgesic activity of ganitri leaf (*Elaeocarpus ganitrus* Roxb) ethanol and methanol extracts in mice (*Mus musculus*).

## METHOD

### Preparation of extracts

In this study, the plants utilized were the leaves. The leaves were collected from Karanggayam, Kebumen in Central Java. The sample was cleaned, cut into the stalk, and went through the air-dried at 25°C

using an oven. Dried simplicial was then mashed with a blender to create powder simplicia and weight in the dried powder. Simplicia powder was later covered in a closed container to avoid sunlight and moisture.

This research used maceration as an extraction method using ethanol and methanol solvent. Two hundred grams of dried powder simplicial were put into maceration container, and ethanol 70% and methanol 70% were added and left for 3 days. The mixture was clarified by filtration using Whatman's filter paper. Maceration of ethanol 70% and methanol 70% were collected and concentrated using a rotary evaporator until achieving thick extract. After that, it was weighted-in and placed in a closed glass container called Ethanol Extract of Ganitri Leaf (EEG) and Methanol Extract of Ganitri Leaf (MEG).

### Phytochemical Screening

Various phytochemical tests were performed to determine alkaloids, flavonoids, tannins, and saponins in extracts.

### Analgesic Activity of Ethanol and Methanol Extracts in Mice

**Experimental Animals.** Male Swiss mice with weights ranging from 20-30 g were used in this study. Animal ethics committee approval was obtained for an animal experiment (Registration number 022101002). The animals were maintained under environmental conditions and had free access to a standard diet and freshwater ad libitum. They were housed in animal cages in an air condition at 25 ± 2° C with 12 hours of light and dark conditions. Prior to the commencement of the experiments, the animals were fasted

for twelve hours but still had access to water.

**Making of Na-CMC 0.5%.** The researchers created Na-CMC by using the following dosage, which was 0.5%. First, weight 0.5 g of Na-CMC, then sprinkled at 100 mL of hot water, stirred vigorously inside mortar for homogeneous until the concentration of Na-CMC was 0.5%.

**Making of 1% of Acetic Acid.** 1 mL of concentrated acetic acid was put into beaker glass. Aquadest was added slowly until it reached volume 100 mL. It was then put into a vial then covered with aluminum foil dan sterilized in autoclave with 121°C for 15 minutes. 0.3 mL acetic acid was inducted into mice with intraperitoneal.<sup>9</sup>

**Testing Ganitri Leaves' Analgesic Activity.** Twenty fours male mice were divided into eight treatment groups. Group I, as the negative control, was given CMC-Na. As the positive control, Group II was given mefenamic acid with 500 mg/kg BW doses. Group III-V was given ganitri leaf ethanolic extract with doses of 100; 200, and 400 mg/kgBW, while group VI-VIII was given ganitri leaf methanolic extract with three doses level respectively 100; 200, and 400 mg/kgBW. All of the treatments were given orally, and then after 30 minutes, they were given acetic acid of 1% doses of 50 mg/kg BW intraperitoneal (i.p). The data of the number of writhes obtained from the analgesic test result was analyzed by counting the protection percentage using the following equation: <sup>10</sup>

$$. \% \text{ protection} = (100 - (P/K \times 100))\%$$

Note:

P = Cumulative number of tested animal writhes after given test compound

K = Median of the cumulative number of negative control tested animal writhes after given test compound

### Statistical Analysis

The result was analyzed using the Shapiro Wilk test to obtain the number of data distribution. According to the test, it was seen that every group has a normal distribution ( $p > 0.05$ ). Afterward, the variant test was conducted, and it resulted in a probability value as much as 0.405 ( $p > 0.05$ ), which showed that the tested data variant was the same. It was continued with a one-way ANOVA test with a 95% trust level, which obtained a probability value as much as 0.000 ( $p < 0.05$ ), which showed that at least the total number of writhes was significant in the two groups. Afterward, Post Hoc analysis was conducted using *Games-Howell* or *LSD* test.

## RESULTS AND DISCUSSION

This research used ganitri leaves powder in the form of ethanolic and methanolic extract. Plant determination was carried out in the Plant Systematics Laboratory of Gadjah Mada University. Based on the determination results, the tested plant was *Elaeocarpus ganitrus* Roxb. Phytochemical screening of both ethanol and methanol extracts showed flavonoids, tannins, and alkaloids, as presented in Table 1.

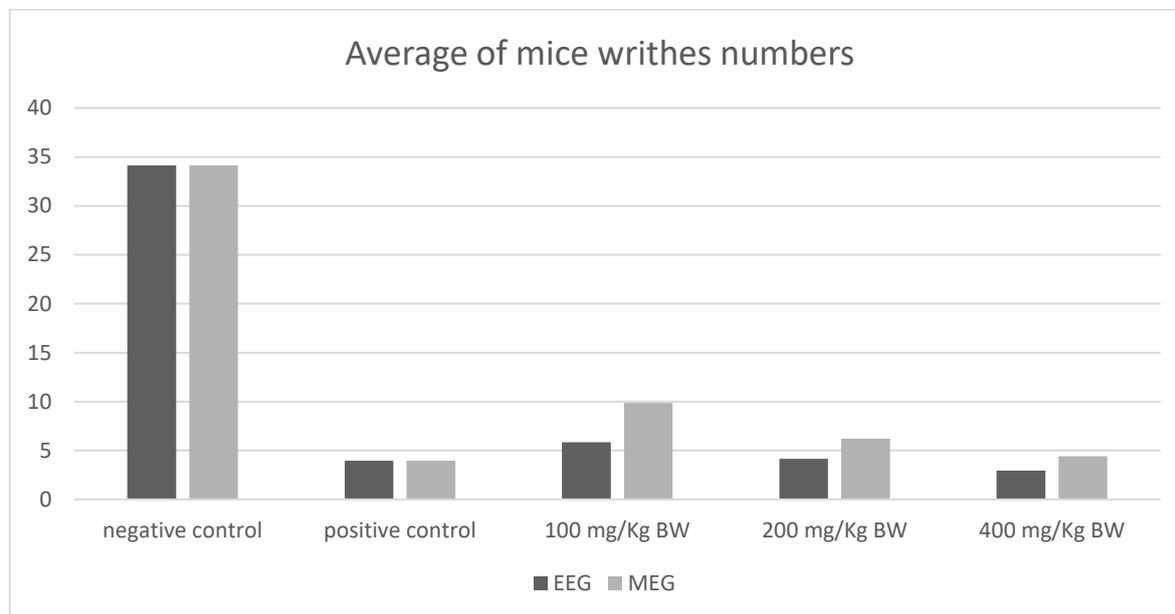
**Table 1.** Phytochemical Screening of Ethanol and Methanol Extracts

Test	Ethanol	Methanol
Flavonoids	+	+
Tannins	+	+
Alkaloids	+	+
Saponin	-	-

(+) and (-) signified presents and absent, respectively

Analgesic activity test in this research used the chemical stimulation method using acetic acid 1% as the pain induction compound. Acetic acid triggers the release of arachidonate from phospholipid tissue.

COX enzyme will change arachidonate acid into prostaglandin, which will stimulate inflammation and pain. The mice showed pain response with writhes.<sup>11</sup>



**Figure 1.** Average graphic of mice writhes numbers

Based on figure one, the highest cumulative number of writhes is shown by the negative control. Ganitri leaf extracts were more likely to decrease writhes compared to the negative control. It showed that at negative control of suspense given by Na-CMC 0.5% did not have an inhibition rate towards writhes or analgesic rate. Mefenamic acid suspense had cumulative of writhes number smaller than EEG at 100 and 200 mg/kg BW and all doses of MEG; thus, the effectiveness of ganitri leaf extract in decreasing the number of writhes was EEG of 400 mg/kg BW dosage, indicating the effect was almost similar to mefenamic acid suspense of 500 mg.

The number of writhes and protection percentage as well as EEG and MEG are presented in Table 2. The Ministry of Health of Indonesia (1991) stated that there was an analgesic activity to the chemical stimulation method shown by the reduction of writhing  $\geq 50\%$  compared to the negative control groups. The mean of writhes of positive control mefenamic acid was significantly different compared to negative control CMC-Na. It showed that mefenamic acid could give analgesic activity to mice induced with acetic acid with percentage inhibition of pain as 88.3%.

**Tabel 2.** Writhe and Ganitri Leaf Protection Percentage on Mice

<i>Test Group</i>	<i>Writhes</i>	<i>Protection Percentage (%)</i>
<i>Negative control (CMC-Na)</i>	34.15	0
<i>Positive control (mefenamic acid)</i>	3.95	88.3
<i>EEG 100 mg/KgBW</i>	5.85	82.8
<i>EEG 200 mg/KgBW</i>	4.15	87.8
<i>EEG 400 mg/KgBW</i>	2.95	91.3
<i>MEG 100 mg/KgBW</i>	9.85	71.2
<i>MEG 200 mg/KgBW</i>	6.2	82.5
<i>MEG 400 mg/KgBW</i>	4.4	87.2

In the treatment, the mean of writhes of ethanolic and methanolic extract of ganitri leaves with doses of 100; 200 and 400 mg/kgBW were significantly different ( $p < 0.05$ ) compared to the negative group of CMC-Na as shown in Table 2. It indicated that the three levels of extract doses could reduce writhing on mice induced with acetic acid. According to The Ministry of Health of Indonesia (1991), all doses of EEG and MEG showed analgesic activity since they showed percentage inhibition of pain to  $> 50\%$  presented in Table 2. This result is similar to the research reported by Nain, Garg, and Dhahiya (2012), revealing that ganitri leaf aqueous extract had analgesic activity, indicating that organic extract both ethanol and methanol could attract compounds that gave analgesic activity. All in all, ganitri leaf extracts contain flavonoids that can act as an analgesic with cyclooxygenase enzyme by reducing the production of prostaglandins with arachidonic acid. Furthermore, flavonoids also block out cytokines, free radicals, and enzymes in inflammation.<sup>43</sup>

## CONCLUSION

The present study showed a significant analgesic effect of both ethanol and methanol extract at 100, 200, and 400

mg/kgBW of ganitri leaves; thus, it had analgesic activity towards acetic acid-induced mice.

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## CONFLICT OF INTEREST

The authors have no conflict of interest

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