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Journal of Natural Sciences and Technology
p-ISSN: 2087-393X | e-ISSN: 2579-5953
http://ejurnal.umri.ac.id/index.php/photon

Analgesic Efficacy Evaluation of Ethanol Extract from African Leaves (*Vernonia amygdalina* Del.) in Acetic Acid-Induced Mice (*Mus musculus*)

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Received 11 November 2022 | Accepted 29 May 2023 | Published 30 November 2024
DOI: https://doi.org/10.37859/jp.v15i1.4246

Keywords:
Vernonia amygdalina Del.; Analgesic effect; Ethanol extract

Abstract. African leaves (*Vernonia amygdalina* Del.) contain many chemical compounds such as tannins, flavonoids, luteolin, coumarins, phenolic acids, lignans, xanthones, anthraquinones, edotides (peptides). African leaves have anti-parasitic, anti-malarial, anti-helminthic, anti-cancer, anticoagulant, analgesic, antipyretic, anti-inflammatory, antioxidant, antidiabetic effects. Tannins are substances in African leaves (*Vernonia amygdalina* Del.) which are capable of causing analgesic effects. In this study, ethanol extract of African leaves was made where the treatment in the group of test animals was differentiated into doses of 100 mg/kgBW, 200 mg/kgBW, 400 mg/kgBW with a positive control in the form of aspirin and a negative control in the form of cooking oil. The results showed that the percentage of analgesic power for the positive control was 87.42%, the dose of ethanol extract of African Leaf 100 mg/kgBW = 52.27%, 200 mg/kgBW = 62.29%, and a dose of 400 mg/kgBW = 81.50 %.

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1. Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage; however, it does not always correspond to the degree of tissue injury observed. Pain is highly individual and influenced by genetic, cultural, age, and gender factors. Failure to assess these complex factors and relying solely on physical examination and laboratory tests may lead to misunderstanding and inadequate pain management, particularly among high-risk groups such as the elderly, children, and patients with communication disorders (Arisetijono et al., 2015).

Pain management commonly relies on modern pharmacological drugs, which often produce various undesirable side effects. In contrast, many communities have long utilized plants as natural sources of traditional medicines, passed down through generations. The World Health Organization

(WHO, 2008) has also recommended the continued use of traditional medicine for maintaining public health, disease prevention, and treatment—provided that it is used safely and effectively.

African leaves (*Vernonia amygdalina* Del.) are among the medicinal plants traditionally used to relieve and control pain. This shrub originates from the African continent and is known for its pharmacological properties, including antioxidant, anticancer, antidiabetic, and analgesic activities (Lubis, 2015, in Aufia et al., 2018). *Vernonia amygdalina* is one of the most widely used species of the Vernonia genus (Toyang & Verpoorte, 2013). It has long been recognized by Chinese communities as a potent medicinal plant that commonly grows in tropical regions, including Indonesia.

According to Ijeh and Ejike (2010), *Vernonia amygdalina* leaves contain several bioactive compounds useful as raw materials for pharmaceuticals, such as saponins, flavonoids, tannins, alkaloids, niacin, anthraquinones, sesquiterpene lactones, and glycosides. Emmanuel et al. (2015) reported that tannins possess analgesic and anti-inflammatory activities.

Previous research on the analgesic effect of *Vernonia amygdalina* leaves was conducted by Miftahul Na'imah (2017), using mice (Mus musculus) induced with acetic acid. The results showed that the leaf infusion at a dose of 1.9 g/kg exhibited an analgesic effect of 4.84%, which was not significantly different from the negative control. Meanwhile, infusions at doses of 4.15 g/kg and 8.3 g/kg demonstrated analgesic activities of 4.46% and 2.45%, respectively—comparable to the positive control, aspirin (65 mg/kg).

Based on this background, the present study aims to investigate the analgesic effect of African leaf (*Vernonia amygdalina* Del.) ethanol extract obtained through maceration in mice (Mus musculus). The maceration method was chosen because *V. amygdalina* contains tannins, which are soluble in organic solvents such as ethanol (Alfa, 2018). Acetic acid was used as the pain-inducing agent because it produces characteristic writhing responses in mice (Domer & Charles, 1971). Mice (Mus musculus) were selected as test animals due to their relatively short life cycle, large litter size, high genetic variability, and ease of handling (Fransius, 2008, in Uswatul H. et al., 2015).

2. The Methods

2.1 Sample collection

Samples of African leaves (*Vernonia amygdalina* Del.) were collected from the Wuryantoro area, Wonogiri, Central Java. The leaves selected were fresh and green, measuring approximately 9–12 cm in length and 3–6 cm in width.

2.2 Sample preparation

The selected *Vernonia amygdalina* leaves were washed thoroughly under running water, cut into small pieces, and air-dried. The leaves were then oven-dried at 50°C until completely dry. Afterward, the dried leaves were ground using a blender to obtain a fine powder (simplicia).

2.3 Maceration process

A total of 200 grams of powdered simplicia was weighed and placed into a beaker glass. Then, 1500 mL of 96% ethanol was added (ratio 1:7.5). The mixture was stirred once every hour and macerated for 18 hours, covered with plastic wrap to prevent evaporation. The macerate was filtered using flannel cloth and filter paper. The resulting filtrate (tincture) was concentrated by evaporating the solvent on a water bath using a pre-weighed porcelain dish until a thick extract was obtained. The extract was then weighed to determine the yield percentage (Depkes RI, 1986).

2.4 Analgesic testing in mice

The analgesic activity was evaluated using the writhing test method. The experimental animals were divided into five treatment groups, each consisting of three mice. Three groups received the ethanol extract of *Vernonia amygdalina* at different concentrations determined through preliminary orientation tests, one group served as the positive control, and another as the negative control.

Mice were fasted for approximately 18 hours before the experiment. The test substances were administered orally 20 minutes prior to intraperitoneal injection of acetic acid to allow sufficient absorption. Pain response was indicated by characteristic writhing behavior, such as stretching of the body, abdominal constriction, or extension of the hind limbs. Observations were conducted for 60 minutes, with counts recorded every 5 minutes. The analgesic effect of the tested extract was determined by the reduction in the number of writhes observed over the 60-minute period.

3. Result and Discussion

Based on the experiment and data analysis of the ethanol extract of African leaves (*Vernonia amygdalina* Del.) as an analgesic agent, the following results were obtained:

3.1 Results of maceration of African leaves

3.1.1 Organoleptic characteristics

Table 1. Result of organoleptic test

Characteristic	Description
Form	Thick extract
Color	Greenish black
Odor	Aromatic
Taste	Bitter

Table 1 shows the organoleptic test result of African leaves extract.

3.1.2 Extraction yield

Maceration of 200 grams of *V. amygdalina* leaf powder using 1500 mL of 96% ethanol produced 14 grams of thick extract, with a yield of 7% (w/w).

3.2. Analgesic test results

The analgesic activity was evaluated using a chemical stimulation method with acetic acid induction, which stimulates pain receptors in peripheral nerves. Aspirin was used as the positive control, as it is a non-narcotic analgesic that acts on pain sites, while cooking oil served as the negative control because it is non-toxic and has no analgesic effect.

To assess the analgesic effect of the ethanol extract of African leaves, the test animals (mice) were divided into five groups, each consisting of five mice. The extract was administered at doses of 100 mg/kg BW, 200 mg/kg BW, and 400 mg/kg BW. The analgesic percentages from these doses were compared with those of the positive control (aspirin, 65 mg/kg BW) and negative control (oil, 25 mL/kg BW).

Pain response was measured by the number of writhes (abdominal contractions) exhibited by the mice during 60 minutes of observation, recorded at 5-minute intervals.

Table 2. Average number of writhes in mice over 1 hour

Mice	Negative control	Positive control	Dose of 400 mg/kg BW	Dose of 200 mg/kg BW	Dose of 100 mg/kg BW
1	55	10	14	25	25
2	67	6	12	23	34
3	66	7	8	22	31

Mean	62.67±3.84	7.67±1.20	11.33±1.76	23.33±0.88	30.00+2.65

Table 2 and 3 are shows average writhes number and the analgesic activity. The data indicate that the number of writhes decreased with increasing extract dose, suggesting a dose-dependent analgesic effect.

Table 3. Percentage of analgesic activity

Treatment	Average analgesic activity (%)
Aspirin 65 mg/kg BW	87.42
Dose of 100 mg/kg BW	52.27
Dose of) 200 mg/kg BW	62.29
Dose of 400 mg/kg BW	81.50

The mean analgesic activity for aspirin (positive control) was 87.42%, which was higher than all doses of the *V. amygdalina* ethanol extract (100, 200, and 400 mg/kg BW). Although lower than aspirin, the extract demonstrated measurable analgesic potential. The data were further analyzed statistically using One-Way ANOVA in SPSS to assess significant differences between treatments at each observation interval.

3.3 Statistical analysis

3.3.1 Normality test

A One-Sample Kolmogorov–Smirnov test was performed to assess data distribution. The significance value obtained was 0.320 (>0.05), indicating that the data were normally distributed.

3.3.2. Homogeneity test

The homogeneity test yielded a significance value of 0.431 (>0.05), indicating that the data were homogeneous across groups.

3.3.3. ANOVA test

The One-Way ANOVA showed a significant difference ($p = 0.000$) among treatment groups, suggesting that at least one treatment group differed significantly from the others. Therefore, a Post Hoc LSD test was conducted.

3.3.4 Post hoc test

The Post Hoc LSD test revealed that the ethanol extract of *V. amygdalina* at 400 mg/kg BW did not differ significantly from aspirin ($p = 0.580 > 0.05$), indicating comparable analgesic activity. In contrast, the 100 mg/kg BW ($p = 0.000 < 0.05$) and 200 mg/kg BW ($p = 0.002 < 0.05$) doses showed significant differences, suggesting weaker analgesic effects compared to aspirin.

3.2 Discussion

This study used *Vernonia amygdalina* leaves because they contain tannins, known for their analgesic properties. The use of traditional medicinal plants such as African leaves has not been widely optimized, and public awareness of their potential health benefits remains limited. Therefore, this research aimed to evaluate the analgesic efficacy of the ethanol extract of *V. amygdalina* prepared via maceration, using male mice (Mus musculus) induced with acetic acid.

The maceration method was chosen because it allows extraction at room temperature with repeated stirring, preserving thermolabile compounds. Tannins, which are abundant in *V. amygdalina*, are highly soluble in organic solvents such as ethanol, making this method suitable.

The writhing test used in this study is a well-established method to evaluate peripheral analgesic activity. Intraperitoneal injection of acetic acid causes irritation and pain, inducing abdominal constrictions (writhing). Similar findings were reported by Na'imah (2017), who used the same method to demonstrate the analgesic effect of *V. amygdalina* infusion.

Organoleptic evaluation of the extract showed a thick, greenish-black extract with an aromatic odor and bitter taste. The analgesic test results demonstrated a clear dose-dependent reduction in the number of writhes, with the 400 mg/kg BW dose showing the greatest effect—approaching that of aspirin.

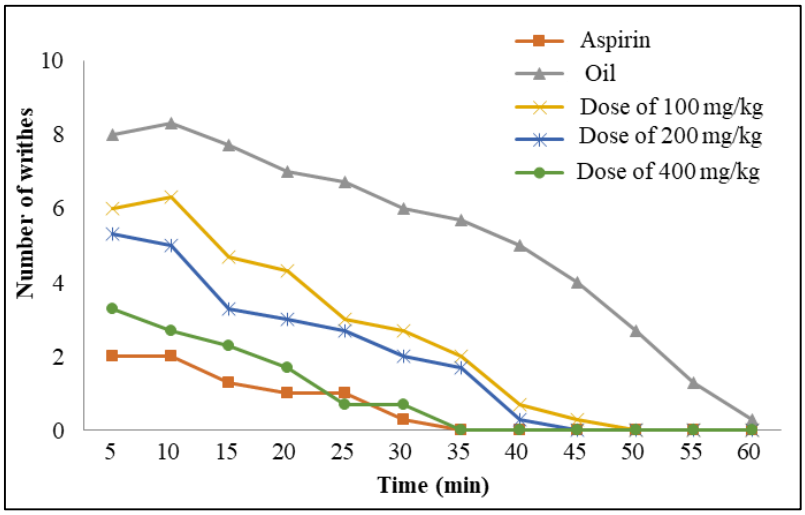

Figure 1. Average writhing response in mice induced by acetic acid at five-minute intervals

The negative control (oil) group exhibited the highest mean number of writhes (62.67), confirming the absence of analgesic effect, while the positive control (aspirin) produced the lowest mean number of writhes (7.67) as shown in Figure 1. The extract at 100, 200, and 400 mg/kg BW produced average writhes of 30.00, 23.33, and 11.33, respectively. The reduction in writhing behavior correlates with the increasing extract dose, indicating that higher doses provide greater pain relief.

The percentage of analgesic activity for the extract doses of 100, 200, and 400 mg/kg BW were 52.27 ± 1.58%, 62.29 ± 3.89%, and 81.50 ± 3.87%, respectively—still lower than aspirin (87.42%), but demonstrating significant analgesic potential. Statistical analysis confirmed that the extract had a significant analgesic effect compared to the negative control ($p = 0.000$), with the 400 mg/kg BW dose being the most effective.

These results are consistent with previous studies (Na'imah, 2017; Delisma et al., 2018) which also found that *V. amygdalina* extracts, at doses of 100–400 mg/kg BW, significantly reduced writhing in mice. The observed analgesic effect may be attributed to the tannins and flavonoids in the extract, which are known to inhibit pain mediators and reduce peripheral nerve sensitivity to inflammatory stimuli.

Conclusion

Based on the results and discussion, it can be concluded that the ethanol extract of African leaves (*Vernonia amygdalina* Del.) exhibits analgesic activity in male mice (Mus musculus L.), indicating its potential as a natural pain-relieving agent. The extract effectively reduced the number of writhes induced by acetic acid in a dose-dependent manner. Among the tested doses, the 400 mg/kg body weight showed the highest analgesic effect, which was comparable to the positive control (aspirin 65 mg/kg body weight). These findings suggest that the ethanol extract of *Vernonia amygdalina* Del. contains bioactive compounds capable of providing significant analgesic effects, supporting its traditional use as a natural remedy for pain management.

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