ANALGESIC POWER OF THE INSOLUBLE FRACTION N-HEXANE OF MELINJO LEAVES (*Gnetum gnemon* L.) IN SWISS MICE (*Mus musculus*)

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Submitted: 6 December 2023 Revised: 11 December 2023 Accepted: 11 December 2023

ABSTRACT

Melinjo (*Gnetum gnemon* L.) *has various secondary metabolites including alkaloids, flavonoids, saponins, and tannins. In its function as an analgesic, flavonoids work to inhibit the cyclooxygenase enzyme which can reduce prostaglandin production and reduce pain. This study aims to determine the analgesic power of the n-hexane insoluble fraction of melinjo leaves* (*Gnetum gnemon* L.) *as an effective analgesic tested on male white mice* (*Mus musculus*) *and to determine the most effective dose as an analgesic. This research was experimental using test animals of 25 male white mice* (*Mus musculus*) *divided into 5 groups each consisting of 5 mice. The negative control group* (*CMC-Na*) *was 0.5%, the positive control group Mefenamic acid dose of 65 mg/KgBW, insoluble fraction hexane group* (*ifh*) *dose of 50 mg/KgBW, 100 mg/KgBW, 200 mg/KgBW. The chemical stimulus was given a dose of 400 mg/KgBW of acetic acid. The parameter measured was the number of wriggles in male white mice* (*Mus musculus*) *every 10 minutes for 60 minutes. The results of the study showed a significant difference between the negative control* (*CMC-Na 0.5%*) *and various doses of insoluble fraction hexane group, while the positive control* (*mefenamic acid 65 mg/KgBW*) *had no difference with ifh doses of 100 mg/kgBB and 200 mg/KgBW. The percentage of analgesic power of ifh at doses of 50 mg/KgBW, 100 mg/KgBW and 200 mg/KgBW was 26.64%, 57.5%, and 58.56% and the percentage of analgesic power of mefenamic acid was 69.67%. In conclusion, ifh doses of 100 and 200 mg/KgBW have effects and potential analgesic power equivalent to mefenamic acid at a dose of 65 mg/KgBW.*

Keywords*: melinjo leaves, analgesic, mice, mefenamic acid*

INTRODUCTION

Pain is a sensation that occurs when the body experiences tissue damage, inflammation, or damage to the nervous system (Chandra et al., 2016). Pain can be relieved using analgesics. Analgesics are drugs that effectively reduce pain. Long-term use of analgesic drugs can cause side effects, such as kidney damage and liver damage, if used in excessive doses (Wardani et al., 2021).

Melinjo leaves (*Gnetum gnemon* L.) are plants that have long been used as traditional medicine in Indonesia and are known to have analgesic effects (Safwan et al., 2016). Melinjo plants (*Gnetum gnemon* L.) contain various secondary metabolites that function as traditional medicinal ingredients. The secondary metabolites found in melinjo plants include alkaloids, flavonoids, saponins, and tannins. Flavonoids function as analgesics and inhibit cyclooxygenase enzymes, which reduce prostaglandin production and pain (Wardani et al., 2021).

From previous research, the analgesic activity test with ethanol extract of melinjo leaves (*Gnetum gnemon* L.) on male white mice (*Mus musculus*) at doses of 6.48 mg/kgBW, 25.92 mg/kgBW, and 51.84 mg/kgBW showed that there was an analgesic effect in male white mice at a dose of 51.84 mg/kgBW (Safwan et al., 2016).

Therefore, based on the background above, the author wanted to examine the analgesic power of polar compounds in melinjo leaves, which were separated by fractionation using an n-hexane solvent, to ensure that the polar compounds in melinjo leaves are effective as analgesics.

RESEARCH METHODS

Materials and Tools

The tools used in this study were aluminum foil, 100 mL beaker glass (Pyrex), stirring rod (Pyrex), maceration vessel, cup, funnel, 500 mL separating funnel (Pyrex), 100 mL measuring cup (Pyrex), animal test cage, mask, water bath, gloves, analytical scales, mouse scales (Soehnle) (0-500 grams), rotary evaporator (IKA), and a stopwatch.

The materials used in this research were Aquadest; melinjo leaves (*Gnetum gnemon* L.); Na-CMC (PT. Bratachem), and n-hexane (PT. Bratachem); mefenamic acid; Ethanol 70% (PT. Bratachem), Glacial acetic acid 100% (Merck), and male white mice (*Mus musculus*).

Research Methods

1. Ethical clearance

   Ethical clearance is proposed to ensure that the treatment administered to the test subjects is ethical. Ethical approval for this research was submitted to the Ethics Commission of the School of Pharmacy Muhammadiyah Cirebon.
2. Collection and provision of goods

   The material used in this research was 2 kg melinjo leaves obtained from Balad Village, Dukupuntang District, Cirebon Regency, West Java, Indonesia.
3. Plant determination

   Plant determination was performed at the IAIN Syekh Nurjati Cirebon Biology Study Laboratory. This identification is used to verify and ensure the correctness of the plant that you wish to use.
4. Animal preparation

   The test animals were 25 healthy male white mice (*Mus musculus*) of the Swiss Webster strain weighing 20−24 grams. The mice were divided into 5 groups consisting of 5 mice each. Group 1 negative control (K-) (Na-CMC 0.5%), positive control group (K+) (mefenamic acid 65 mg/kgBW) treatment group was divided into 3 groups: ifh50 (dose 50 mg/kgBW, ifh100 (dose 100 mg/kgBW), ifh200 (dose 200 mg/kgBW]).
5. Melinjo leaves simplicia

   The plant material used was 2 kg of melinjo leaves, made by collecting raw materials, wet sorting them, washing them with running water, draining them, and drying them in an oven at 40°C for 24 hours. Then, the dried leaves were sorted again to remove the dirt left behind during the drying process. Next, the simplicia is mashed using a blender and sifted to obtain simplicia powder. The obtained powder was stored in a clean closed container.
6. Extraction of melinjo leaves

   Melinjo leaves simplicia (200 g) were placed into a vessel, and 75 parts of filter liquid (70% ethanol) were added to 1500 mL, covered, and left in the vessel for 5 days protected from light. Subsequently, the solution was filtered and squeezed. The dregs were washed with 25 parts of 500 mL filter fluid (70% ethanol). Transfer to a closed vessel, leave in a cool place protected from light, and leave for 2 days. Then, the precipitate was separated, and the macerate was concentrated using a rotary evaporator at a temperature of 50°C. Evaporation was performed using a water bath until a thick extract of Melinjo leaves was obtained.
7. Preparation of n-hexane insoluble fraction of melinjo leaves

   Five grams of concentrated melinjo leaf extract were added to 30 mL of water and then fractionated with n-hexane solvent with a replication of 3 × 30 mL. The extract was concentrated using a water bath until it became a thick extract.

8. Phytochemical screening

   Phytochemical screening was carried out on the extract and n-hexane-insoluble fraction of melinjo leaves (*Gnetum gnemon* L.) to check for the presence of secondary metabolites. In general, these compounds include Alkaloids, Flavonoids, Tannins, and Saponins (Firmansyah et al., 2022).

9. Treatment animal test

   Experimental animals were first adapted for 7 days and fasted for ± 16 hours without being fed but still given water. Mice were weighed and divided into 5 groups. The positive control group was mefenamic acid 65 mg/kgBW, the negative control group was given Na-CMC 0.2 mL/20 grams BW, the ifh50 group was given the insoluble fraction of n-hexane melinjo leaves at a dose of 50 mg/kgBW, the ifh100 group was given the insoluble fraction n-hexane of melinjo leaves at a dose of 100 mg/kgBW, the ifh200 group was given the insoluble n-hexane fraction of melinjo leaves at a dose of 200 mg/kgBW. Each group was orally injected. After 5 minutes of administration, 4% acetic acid was intraperitoneally administered to each mouse. The number of wriggles that occurred every 10 minutes for 60 minutes.

Data Analysis

The research data obtained were then processed and analyzed using the one-way ANOVA statistical test with the LSD post hoc test.

RESULTS AND DISCUSSION

Plant Determination

Determination was carried out in the biology laboratory of IAIN Syekh Nurjati Cirebon to compare one plant with another and determine the correctness of the samples used in the study (Klau & Hesturini, 2021).

Melinjo Leaf Extraction

The extraction process was carried out in a cold environment using the maceration method. This method is performed so that the active substance is not damaged if it contains metabolized substances (Handayani & Hastuti, 2023). Maceration was carried out using 70% ethanol solvent because ethanol can attract more active compounds and has a low boiling point of 79°C; therefore, less heat is required for the concentration process (Hasanah & Dede, 2020). The maceration results are evaporated using a vacuum rotary evaporator to concentrate the solution, which consists of dissolved substances that do not easily evaporate at low temperatures. The mixture was evaporated in a water bath until a thick extract was obtained in the form of a dark green liquid with a yield of 19.02%.

N-Hexane Insoluble Fraction of Melinjo leaves

Fractionation was then carried out to separate the compounds based on different polarity levels in the two solvents (Pratiwi et al., 2016). Fractionation was carried out using two solvents with different polarity levels: nonpolar n-hexane and polar water. Fractionation of melinjo leaf extract produced 3 layers: the top layer was the n-hexane fraction, the middle layer was the interphase, and the bottom layer was the n-hexane insoluble fraction. This can be influenced by the amphiphilic nature of phospholipids and other particles in melinjo leaves (Ruiz et al., 2022). Melinjo leaves contain fatty acids such as oleic acid, linoleic acid, palmitic acid, and stearic acid (Haryani et al., 2016).

Phytochemical Screening Results

Table I. Results of Phytochemical Screening of Melinjo Leaves Extract (*Gnetum gnemon* L.)

No	Testing	Reagents	Results	Information
1.	Alkaloids	Dragendorff	Orange precipitate	Positive
2.	Flavonoids	Mg metal + Concentrated HCl	Orange solution	Positive
3.	Tannin	$FeCl_3$	Blackish green solution	Positive
4.	Saponin	Aquadest + HCl 1N	Foamy	Positive

Phytochemical screening was performed to qualitatively determine the secondary metabolite compounds found in the leaves. In melinjo leaves (*Gnetum gnemon* L.), the results of phytochemical screening tests showed that melinjo leaf extract contains flavonoids, tannins, saponins, and alkaloids.

Table II. Phytochemical Screening Results of the Insoluble Fraction of n-hexane Melinjo Leaves (*Gnetum gnemon* L.)

No	Testing	Reagents	Results	Information
1	Alkaloids	Dragendorff	Orange precipitate	Positive
2	Flavonoids	Mg metal + Concentrated HCl	Orange solution	Positive
3	Tannin	$FeCl_3$	Blackish green solution	Positive
4	Saponin	Aquadest + HCl 1N	Foamy	Positive

Phytochemical screening was performed to qualitatively determine the secondary metabolite compounds found in the leaves. In melinjo leaves (*Gnetum gnemon* L.), the results of phytochemical screening tests showed that the n-hexane-insoluble fraction of melinjo leaves contained flavonoids, tannins, saponins, and alkaloids.

Animals Preparations

The test animals in this study were male white mice, and ethical approval for using test animals was obtained from document No. 031.VI.23.4040.KEPK.STFMC from the Health Research Ethics Committee of the School of Pharmacy Muhammadiyah Cirebon, Cirebon Regency, West Java, Indonesia.

Analgesics Test Results

The analgesic activity of the hexane-insoluble fraction of melinjo leaves on male white mice using Na CMC as the extract solvent and as a negative control was used to compare whether there was an analgesic effect against the positive control (Lara et al., 2021). Evaluation of analgesic activity using the writing test. The principle of this technique is to measure the reduction in the number of squirms resulting from the administration of an inductor, 4% acetic acid (Firmansyah et al., 2019). Intraperitoneal administration of 4% acetic acid induces writhing pain in mice. The writhing condition is characterized in test animals by the activity of both legs of the test animal being pulled back and contractions of the abdominal wall.

Induced acetic acid as an inductor of pain stimulation in test animals using the writhing test method is suitable for detecting central and peripheral analgesia. Administration of acetic acid releases prostaglandins and sympathomimetic mediators such as PGE2 and PGFalfa. The administration of flavonoid compounds contained in ifh can inhibit the synthesis or action of prostaglandins after induction by acetic acid (Firmansyah et al., 2022).

Table III. Analgesic Activity of the Insoluble Fraction N-Hexane of Melinjo Leaves (*Gnetum gnemon* L.) using the Chemical Stimulation Method (Writhing Test)

Groups	Cumulative Number of Stretches (±SD)	Stretch Protection (%)
K(-)	9,97±3,06	-
K(+)	3,1±0,97[a]	69,67%
ifh50	7,4±2,34[a]	26,64%
ifh100	4,2±1,92[a]	57,50%[b]
ifh200	4,17±1,59[a]	58,56%[b]

Note: K+ (positive control); K- (negative control); ifh50, ifh100, ifh200 (insoluble fraction n-hexane of melinjo leaves dose 50 mg/kgBW, 100 mg/kgBW, and 200 mg/kgBW)
[a], p<0,05: there was a significant difference in the negative control
[b], p>0,05: there was no significant difference in the positive control

The results of the study showed that the negative control group had the highest average number of wriggles (9.97 ± 3.06), which came from the average number of test animals writhing for one hour. This is because the negative control group did not contain any active ingredients in its administration. The only solvent administered was Na-CMC 0.5%. The results of statistical analysis using the ANOVA test with post hoc LSD showed that the cumulative number of movements of the ifh50 (7.4 ± 2.34), ifh100 (4.2 ± 1.92), and ifh200 (4.17 ± 1.59) groups differed significantly from the negative control group. However, when comparing the number of writhes with the positive control, namely mefenamic acid, none of the treatment groups (ifh50, ifh100, and ifh200) showed significant differences. These results show that the hexane-insoluble fraction of melinjo leaves (*Gnetum gnemon* L.) can reduce the pain stimulation induced by acetic acid.

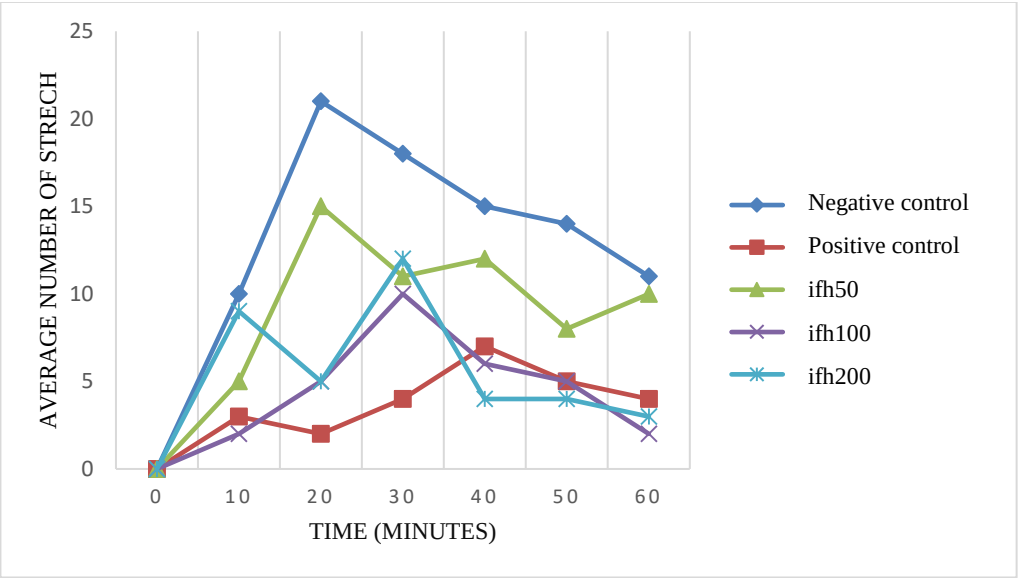

Figure 1. Graph of the effect of the dose of the insoluble fraction of n-hexane of melinjo leaves (*Gnetum gnemon* L.) on the writhing response in minutes in test animals (*Mus musculus*)

Stretch protection and percentage analgesic power are shown in Table III. The positive control group (methampyrone dose 65 mg/kgBW) had the highest percentage value (69.67 %), while the highest percentage of analgesic power in the treatment group was observed in the ifh200 group (58.56 %). In Figure 1, it can be concluded that the greater the fraction

concentration, the greater the potential for protection in reducing pain stimulation, while the negative control was above the positive control graph and all treatments. This proves that Na-CMC 0.5% as a negative control, is unable to inhibit pain.

The flavonoid content in melinjo leaves has a significant potential for inhibiting pain stimulation. Flavonoids are strong antioxidants that exert analgesic effects by inhibiting the action of the cyclooxygenase enzyme, thus reducing prostaglandin production by arachidonic acid. As prostaglandins are pain mediators, inhibiting prostaglandin activity can reduce pain stimulation (Salim et al., 2017).

CONCLUSION

The insoluble fraction n-hexane of melinjo leaves (*Gnetum gnemon* L.) at doses of 50, 100, and 200 mg/kgBW can reduce acetic acid-induced pain stimulation in male white mice. The best analgesic protection percentage was ifh at a dose of 100 mg/kgBW (57.50%) and a dose of 200 mg/kgBW (58.56%) which had a protection percentage that was no different from mefenamic acid at a dose of 65 mg/kgBW. Melinjo leaves can be used as an alternative to natural analgesic drugs.

ACKNOWLEDGMENT

The author would like to thank the Institute for Research and Community Service, School of Pharmacy Muhammadiyah Cirebon, for funding research and publication.

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