3rd Makassar International Conference in Pharmaceutical Science (MICPS) 2025
Jurnal Fitofarmaka Indonesia, 2025; 12(3) xx-xx
http://jurnal.farmasi.umi.ac.id/index.php/fitofarmakaindo/index

Analgesic Effect of Ethanol Extract *Myristica fragrans* Houtt Using the Hot-Plate Method on Mice

Hendra Herman[123], Aulia Wati[12*], Zalsa Safirah Hijrana-A[1]
[1]Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, South Sulawesi, Indonesia
[2]Faculty of Pharmacy, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia
[3]Ibnu Sina Hospital YW UMI, Makassar, South Sulawesi, Indonesia

Article info
History
Submission: 09-10-2025
Review: 09-11-2025
Accepted: 17-11-2025

***Email:**
aulia.wati@umi.ac.id

DOI: 10.33096/jffi.v12i3.1387

Keywords:
Myristica fragrans Houtt*; analgesic; hot-plate method; mice model*

Abstract
Pain is a subjective and complex physiological response of the body to tissue damage. Leaves of *Myristica fragrans Houtt* contain active compounds such as flavonoids, alkaloids, tannins, and myristicin, which may affect analgesic properties. The aim of study aims to evaluate the analgesic effect and effective dose of leaf ethanol extract Myristica fragrans Houtt using the hot-plate method. A total of thirty mice were randomly divided into 6 groups, namely negative control (Na-CMC), positive control (paracetamol), and four treatment groups with extract doses of 200, 300, 400, and 500 mg/kgBW. Pain responses were observed through jumping and paw licking behavior at 30-minute intervals over 120 minutes, with cut-off 5 minutes. Data were analyzed statistically using Kruskal-Wallis with a follow-up Mann-Whitney test. The results showed that all doses of ethanol extract Myristica fragrans Houtt were significantly different ($p<0.05$) compared the negative and positive groups. All variations in the dose of leaf ethanol extract had an analgesic effect that was not significantly different ($p>0.005$). The analgesic effectiveness of ethanol extract at a dose of 500 mg/kg BW was the greatest at 55.36%. The research results concluded that ethanol extract of *Myristica fragrans Houtt* had an analgesic effect and the most effective dose is 500 mg/kg BW.

I. Introduction

Pain is an unpleasant sensory and emotional experience that is subjective, complex, and involves physiological responses to tissue damage (DiPiro *et al.*, 2020). According to the World Health Organization (WHO), chronic pain-such as back pain, headaches, and joint pain-is a common complaint across various countries. Data from the 2018 National Basic Health Research (Riset Kesehatan Dasar) reported a musculoskeletal disease prevalence of 11.9%, with back pain ranging from 7.6% to 37%, joint pain at 7.3%, and injury-related pain at 9.2% (Emril, Basar and Kurniawan, 2018; Kumbea, Asrifuddin and Sumampouw, 2021).

Pain management is commonly achieved through the administration of synthetic analgesics; however, prolonged use may lead to adverse effects such as gastrointestinal disturbances, hepatotoxicity, and nephrotoxicity. Consequently, the search for safer and equally effective analgesic alternatives derived from natural sources has become increasingly important (Tjay and Rahardja, 2007).

Nutmeg (*Myristica fragrans* Houtt.) has long been used in traditional medicine, particularly its seeds and essential oil, which possess antiseptic, antirheumatic, and analgesic properties (Puspa, Syahbanu and Wibowo, 2017; Pareta, 2023). However, the leaves of nutmeg remain underexplored scientifically, despite being known to contain bioactive compounds such as flavonoids, alkaloids, triterpenoids, tannins, and myristicin, which exhibit potential anti-inflammatory and analgesic effects (Pratiwi, Noorlaela and Mahyuni, 2019; Sri *et al.*, 2023).

Previous studies have demonstrated analgesic activity in other parts of the nutmeg plant, including the fruit pulp, seeds, and oleoresin (Nur and Rahman, 2020; Mayefis *et al.*, 2021; Sri *et al.*, 2023). Nevertheless, to date, no research has specifically evaluated the analgesic effect of nutmeg leaf ethanol extract using the hot-plate method. This method assesses central analgesic activity by measuring latency to thermal stimuli, making it suitable for testing compounds that act on the central nervous system.

Based on these considerations, this study was conducted to evaluate the analgesic effect and determine the effective dose of leaf ethanol extract of *Myristica fragrans Houtt* in mice using the hot-plate method, as part of an effort to develop safe and pharmacologically promising natural analgesic alternatives.

II. Research Method

II.1 Instruments and Materials

This study utilized various laboratory instruments, including a cannula, syringe, stopwatch, analytical balance (Ohaus), hot-plate apparatus (IKA®), stirring rod, mortar and pestle, volumetric flask, as well as animal cages and weighing scales. The materials used comprised filter paper, distilled water, leaves (*Myristica fragrans Houtt*), 96% ethanol, 1% sodium carboxymethyl cellulose (Na-CMC), Fasidol® acetaminophen tablets (500 mg) manufactured by PT. Ifars Pharmaceutical Laboratories, and standard animal feed.

II.2 Preparation and Extraction of Nutmeg Leaf Simplicia

Nutmeg leaves (*Myristica fragrans Houtt.*) were collected from Buntu Kunyi Village, Suli Subdistrict, Luwu Regency, South Sulawesi. The samples were rinsed with running water, wet-sorted, and air-dried at room temperature. Once dried, the leaves were re-sorted and ground into simplicia powder. A total of 500 g of powder was macerated with 3 L of 96% ethanol, stirred for 15 minutes, and left to stand for 72 hours. The filtrate was filtered three times and then concentrated using a rotary evaporator at 50 °C until a thick extract was obtained (Agustien *et al.*, 2024).

II.3 Selection and Preparation of Experimental Animals

A total of 30 male white mice weighing 20–30 g were used as experimental subjects and divided into six treatment groups, each consisting of five animals. Prior to treatment, the mice were housed in standard cages and provided with food and water ad libitum during the acclimatization period.

II.4 Preparation of 1% Na-CMC Suspension (b/v)

One gram of sodium carboxymethyl cellulose (Na-CMC) was weighed and placed into a beaker. It was gradually mixed with 50 mL of distilled water while stirring until homogeneous, then the volume was adjusted to 100 mL with additional distilled water (Amir *et al.*, 2023).

II.5 Preparation of Paracetamol Suspension

Ten tablets of paracetamol were weighed and ground into powder, then transferred into a volumetric flask and dissolved in 1% Na-CMC. The volume was adjusted to 10 mL to obtain a uniform suspension (Sulistiawati, Rahmana and Ro'uf, 2024).

II.6 Preparation of Nutmeg Leaf Ethanol Extract Suspension

Nutmeg leaf ethanol extract (EEDP) was dissolved in 1% Na-CMC to prepare four dosage variations: 200, 300, 400, and 500 mg/kgBB. Each dose was formulated by dissolving 60, 90, 120, and 150 mg of EEDP in 10 mL of 1% Na-CMC, followed by homogenization (Syamsul, Andani and Soemarie, 2016).

II.7 Analgesic Effect Test

Thirty male white mice weighing 20–30 g were randomly assigned to six treatment groups, each consisting of five animals. After fasting for 8 hours with free access to water, the mice were administered treatments orally: 1% Na-CMC (negative control), paracetamol 200 mg/kgBB (positive control), and EEDP at doses of 100, 200, 400, and 500 mg/kgBB. The analgesic effect was evaluated using the hot-plate method, in which each mouse was placed on a metal surface maintained at ±52 °C and observed for 120 minutes. Response time was recorded as the latency to pain reaction, indicated by paw licking or jumping off the hot plate, with a cut-off time of 5 minutes.

II.8 Calculation of Analgesic Effectiveness

The analgesic effectiveness of each treatment was evaluated based on the pain responses exhibited by the test animals using the hot-plate method. To quantify the level of analgesic activity and effectiveness, a quantitative approach was employed by calculating the percentage of analgesic activity relative to the negative control, as well as the percentage of analgesic effectiveness compared to the positive control. The calculation formulas are presented as follows Formula 1 for % analgesic activity and Formula 2 for % analgesic effectiveness.

% Analgesic Activity

$$= 100 - \frac{\text{Mean response of treatment group}}{\text{Mean response of negative control}} \times 100\% \quad (1)$$

% Analgesic Effectiveness

$$= \frac{\text{\% Analgesic Activity of Treatment Group}}{\text{\% Analgesic Activity of the Positive Control Group}} \times 100\% \quad (2)$$

II.9 Data Analysis

The cumulative pain response data (paw licking and jumping) obtained from the experiment were statistically analyzed using the Kruskal-Wallis test, followed by post hoc analysis with the Mann-Whitney test. This non-parametric approach was selected due to the nature of the data distribution and sample size, allowing for accurate comparison of analgesic effects across treatment groups.

III. Results and Discussion

This study began with the selection of nutmeg leaves (*Myristica fragrans* Houtt.) as the active material, based on their diverse bioactive compound content and limited prior utilization. The leaves were dried and extracted using maceration for three consecutive days with 96% ethanol as the solvent. This method was chosen for its simplicity and its ability to preserve thermolabile active compounds. The extraction process was conducted at room temperature to minimize degradation,

ensuring that the resulting extract retained optimal pharmacological properties.

Following extraction, the next phase involved preparing the test animals—male mice (*Mus musculus*) aged 2–3 months and weighing 20–30 grams. Mice were selected due to their biological characteristics that closely resemble humans, and male mice tend to exhibit more stable pain responses. Prior to treatment, the animals underwent a seven-day acclimatization period to adapt to the laboratory environment and reduce stress that could affect experimental outcomes. The experiments were conducted according to the ethical guidelines approved by the Komite Etik Penelitian (KEP) Universitas Muslim Indonesia, Makassar, Indonesia with number 412/A.1/KEP-UMI/VII/2025 and registration number is UMI012505319.

The analgesic effect was assessed using the hot-plate method, which relies on thermal stimulation at a temperature of 50–55°C. This method was selected for its ability to evaluate centrally acting analgesic activity without causing injury to the test animals. The type of pain assessed was acute nociceptive pain, triggered by thermal stimuli, which is relevant for evaluating compounds that influence pain thresholds.

Each mouse was placed on the heated plate surface, and pain-related behaviors such as paw licking, trembling, grooming, and jumping were observed as indicators of pain threshold. Observations were conducted over a 120-minute period at 30-minute intervals. A reduction in the frequency of pain behaviors indicated that the administered compound was capable of elevating the pain threshold, thereby demonstrating analgesic potential.

The test animals were divided into six groups: a negative control (1% Na-CMC), a positive control (paracetamol 500 mg/kgBW), and four treatment groups receiving nutmeg leaf ethanol extract at graded doses of 200, 300, 400, and 500 mg/kgBW. The 300 mg/kgBW dose was used as a reference based on previous studies indicating optimal central nervous system efficacy. Dose variation was employed to evaluate the range of effectiveness and to identify the most promising dose for herbal analgesic development.

Observations were recorded at 30, 60, 90, and 120-minutes post-administration, noting the onset of pain-related behaviors as indicators of pain threshold. These data were compiled into tables to facilitate comparison across control and treatment groups, and to assess the consistency of analgesic effects at varying extract doses.

Table 1. Mean pain response across treatment groups

Treatment Groups	30 minutes	60 Minutes	90 minutes	120 minutes	Total
Na-CMC	25,5±7,38	18,25±9,42	17,5±4,79	17,25±4,57	78,5±9,03
Paracetamol	6,0±4,76	3,0±2,16	2,5±1,91	10,75±1,89	22,5±3,20
EEDP 200 mg/kgBB	24,0±3,91	13,75±2,05	9,25±0,95	6,75±1,70	53,75±4,99
EEDP 300 mg/kgBB	22,25±6,07	15,0±2,94	8,0±1,82	6,5±2,38	51,75±4,64
EEDP 400 mg/kgBB	20,75±2,21	14,5±3,41	8,0±2,0	6,0±2,16	49,25±4,43
EEDP 500 mg/kgBB	20,25±2,06	14,0±3,36	7,25±1,70	6,0±1,82	47,5±3,41

Note: EEDP = Nutmeg leaf ethanol extract

Based on Table 1, the negative control group (1% Na-CMC) exhibited consistently high pain response frequencies throughout the observation period (78.5 ± 9.03), consistent with its inert nature as a suspending agent without analgesic properties (Firdaus and Priamsari, 2019; Wardani *et al.*, 2021). In contrast, the positive control group (paracetamol 500 mg/kgBB) showed the lowest pain response (22.5 ± 3.20), reflecting its central mechanism of action through inhibition of prostaglandin synthesis via COX enzyme blockade and activation of the descending serotonergic system (Katzung, Master and Trevor, 2014; Hidayat, Harahap and Villyastuti, 2017).

Treatment groups receiving ethanol extract of nutmeg leaves (EEDP) at graded doses demonstrated a gradual reduction in pain response. The 500 mg/kgBB dose produced the most pronounced effect, with a mean pain response of 47.5, indicating the highest analgesic potential among the extract-treated groups. A lower observed pain response corresponds to a stronger analgesic effect exerted by the test preparation (Desiani *et al.*, 2022).

The mean pain response data were subsequently used to calculate the percentage of analgesic potency and analgesic effectiveness. Percentage of analgesic potency aimed at evaluating the extent to which EEDP reduced pain through decreased response frequency or increased latency to thermal stimulation (Familia *et al.*, 2024). Percentage of analgesic effectiveness aimed to assess how optimally the extract reduces pain compared to the standard analgesic (Azizah, Putra and Alda, 2024). There result of calculation are presented in Table 2.

Table 2. Percentage of analgesic activity

Treatment Groups	% Analgetic Potency	% Analgetic Effectiveness
Negative Control	-	-
Positive Control[a]	71,34%	-
EEDP 200 mg/kgBW[ac]	31,53%	44,19%
EEDP 300 mg/kgBW[ac]	34,08%	47,77%
EEDP 400 mg/kgBW[ac]	37,27%	52,24%
EEDP 500 mg/kgBW[ac]	39,50%	55,36%

note: EEDP = Nutmeg leaf ethanol extract; a: p<0,05 compared to Negative Control (Mann-Whitney test); b: p>0,05 compared to negative control (Mann-Whitney test); c: p<0,05 compared to Positive Control (Mann-Whitney test); d: p>0,05 compared to Positive Control (Mann-Whitney test)

Based on Table 2, the positive control group (paracetamol) exhibited the highest percentage of analgesic activity at 71.34%. Among the treatment groups, the ethanol extract of nutmeg leaves (EEDP) at a dose of 500 mg/kgBB produced the strongest effect, with an analgesic activity percentage of 39.5%. The results of analgesic effectiveness percentage, the ethanol extract of nutmeg leaves (EEDP) at a dose of 500 mg/kgBB demonstrated the highest value among all treatment groups, reaching 55.36%. This indicates that the extract at this dose was capable of producing a notable analgesic effect, approaching the efficacy of the standard drug. These findings support the potential of EEDP as a natural analgesic agent.

Pain response data for each group were analyzed using SPSS version 31. The data analysis was conducted using the Kruskal-Wallis test followed by the Mann-Whitney post hoc test to determine significant differences between groups are presented in Table 2.

The Mann-Whitney test results revealed that the negative control group (Na-CMC) differed significantly from both the positive control (paracetamol) and all EEDP-treated groups, with p-values < 0.05. This confirms that Na-CMC lacks analgesic properties and functions solely as a suspending agent. Furthermore, paracetamol showed significant differences when compared to all EEDP doses, indicating its superior analgesic potency. In contrast, no significant differences were observed among the various EEDP doses (p > 0.05), suggesting that all tested concentrations exerted relatively similar analgesic effects. Nevertheless, as shown in Table 2, the 500 mg/kgBB dose consistently produced the lowest mean pain response and the highest analgesic effectiveness percentage at 55.36%.

This effectiveness is presumed to be attributed to the presence of active secondary metabolites such as alkaloids, flavonoids, tannins, and steroids. These compounds are known to inhibit cyclooxygenase and phospholipase enzymes, thereby reducing prostaglandin synthesis and suppressing pain signaling pathways (Kharisma-P *et al.*, 2020; Debeturu *et al.*, 2022). The combined activity of these constituents positions the 500 mg/kgBB dose as the most promising candidate, although its potency remains inferior to that of paracetamol as the standard reference.

IV. Conclusions

Based on the research findings and data analysis, the ethanol extract of nutmeg leaves (*Myristica fragrans Houtt*) demonstrated analgesic effects in male mice as assessed by the hot-plate method. Among the tested doses, 500 mg/kgBB exhibited the highest effectiveness in reducing pain responses, with an analgesic effectiveness percentage of 55.36%. These results indicate that nutmeg leaf extract holds promising potential for development as a natural analgesic candidate.

V. Acknowledgment

The authors thank the Faculty of Pharmacy, Universitas Muslim Indonesia, for research support and laboratory facilities. Appreciation is also extended to all individuals who assisted in proofreading and material provision.

References

Agustien, G.S. *et al.* (2024) 'Uji Mutu Ekstrak Etanol Daun Pala Muda Dan Daun Tua (Myristica fragans)', in *1st Perjuangan Nature Pharmaceutical Conference*. Tasikmalaya: Program Studi Farmasi Universitas Perjuangan Tasikmalaya, pp. 64–71.

Alda Nur Azizah, R. and Putra, B. (2024) 'Pengujian aktivitas analgetik (Portulaca oleracea L.) pada mencit putih (Mus musculus) menggunakan metode hot plate', *Jurnal Farmasi Desember*, 16(2).

Amir, M.N. *et al.* (2023) 'Studi In Vivo Ekstrak Etanol Kulit Buah Nangka (Artocarpus heterophyllus L.) Sebagai Kandidat Obat Analgetik Terhadap Model Hewan Uji Mecit (Mus musculus)', *Jurnal Mandala Pharmacon Indonesia*, 9(1), pp. 139–147. Available at: https://doi.org/10.35311/jmpi.v9i1.338.

Azizah, R.N., Putra, B. and Alda, A. (2024) 'Pengujian Aktivitas Analgetik Infusa Herba

Krokot (Portulaca oleracea L.) Pada Mencit Putih (Mus musculus) Menggunakan Metode Hot Plate', *As-Syifaa Jurnal Farmasi*, 16(2), pp. 163–170. Available at: https://doi.org/https://doi.org/10.56711/jifa.v16i2.1274.

Desiani, E. *et al.* (2022) 'Uji Aktivitas Analgesik Ekstrak Daun Mangrove (Rhizophora mucronata) Pada Mencit Yang Diinduksi Asam Asetat Dengan Metode Writhing Reflex', *Cendekia Journal of Pharmacy*, 6(2), pp. 307–317. Available at: http://cjp.jurnal.stikescendekiautamakudus.ac.id.

DiPiro, J.T. *et al.* (2020) *Pharmacotherapy: a Pathophysiologic Approach*. 11th ed. McGraw Hill Medical.

Emril, D.R., Basar, A.A. and Kurniawan, H. (2018) 'Pola Penatalaksanaan Nyeri Neuropatik di Pusat Pelayanan Kesehatan Primer Di Kota Banda Aceh', *Jurnal Sinaps*, 1(3), pp. 78–91.

Familia, D. *et al.* (2024) 'Uji Aktivitas Analgetik Ekstrak Etanol Daun Ramania (Bouea macrophylla Griffth) Terhadap Mencit Putih (Mus musculus) Dengan Metode Writhing Test', *Parapemikir: Jurnal Ilmiah Farmasi*, 13(1), pp. 44–52. Available at: https://doi.org/https://doi.org/10.30591/pjif.v13i1.5756.

Firdaus, N.K. and Priamsari, M.R. (2019) 'Uji Daya Analgesik Ekstrak Etanolik Kulit Terong Belanda (Solanum betaceum Cav.) Ada Mencit Jantan Galur Swiss Yang Diinduksi Asam Asetat 1%', *Jurnal Akademi Farmasi Prayoga*, 4(2), p. 2019.

Hidayat, A.P., Harahap, M.S. and Villyastuti, Y.W. (2017) 'Perbedaan Antara Parasetamol Dan Ketorolak Terhadap Kadar Substansi P Serum Tikus Wistar Sebagai Analgesik', *Jurnal Anestesiologi Indonesia*, IX(1), pp. 38–49. Available at: https://doi.org/https://doi.org/10.14710/jai.v9i1.19853.

Katzung, B.G., Master, S.B. and Trevor, A.J. (2014) *Farmakologi Dasar & Klinik*. 12th ed. Jakarta: Penerbit Buku Kedokteran EGC.

Krisna Wahyuni, H. and Hesturini, R.D. (2020) 'Uji aktivitas analgesik daun trembesi (Samanea saman (Jacq.) Merr.)', *Jurnal Wiyata*, 7(2).

Kumbea, N.P., Asrifuddin, A. and Sumampouw, O.J. (2021) 'Keluhan Nyeri Punggung Bawah Pada Nelayan', *Journal of Public Health and Community Medicine*, 2(1), pp. 21–26. Available at: https://doi.org/https://doi.org/10.35801/ijphcm.2.1.2021.33580.

Mayefis, D. *et al.* (2021) 'Uji Efek Stimulan Sistem Saraf Pusat Dari Ekstrak Etanol Biji Pala (Myristica fragrans H.) Pada Mencit Putih Jantan', *JULI*, 1(2), pp. 75–86. Available at: https://journal.amikveteran.ac.id/index.php/.

Pareta, D.N. (2023) 'Potensi Minyak Atsiri Biji Pala Dalam Pengembangan Bahan Alam sebagai Sumber Obat', *Majalah InfoSains*, 4(1), pp. 1–3.

Nur, A. and Rahman, I. (2020) 'Uji Efek Analgetik Ekstrak Etanol Buah Pala Terhadap Tikus Putih', *Kieraha Medical Journal*, 2(2), pp. 268–5912. Available at: https://ejournal.unkhair.ac.id/index.php/kmj.

Pratiwi, A., Noorlaela, E. and Mahyuni, S. (2019) 'Uji Daya Hambat Sediaan Sabun Cair Ekstrak Daun Pala (Myristica fragrans houtt) Terhadap Propionibacterium acnes dan Staphylococcus aureus', *Ekologia: Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*, 19(2), pp. 80–88. Available at: https://doi.org/10.33751/ekol.v19i2.1649.

Puspa, O.E., Syahbanu, I. and Wibowo, M.A. (2017) 'Uji Fitokimia Dan Toksisitas Minyak Atsiri Daun Pala (Myristica fragans Houtt) Dari Pulau Lemukutan', *Jurnal Kimia Khatulistiwa*, 6(2), pp. 1–6.

Sri, S.S.G. *et al.* (2023) 'Formulasi Sediaan Balsem Stik Oleoresin Biji Pala (Myristica fragrans Houtt)', *Jurnal Ilmiah Pharmacy*, 10(2), pp. 117–124.

Sulistiawati, N., Rahmana, R.I. and Ro'uf, A. (2024) 'Perbandingan Aktivitas Analgesik Parasetamol Dan Antalgin Pada Mencit Putih Jantan (Mus Musculus) Dengan Metode Writhing Test', *Jurnal Ilmiah Farmasi Attamru (JIFA)*, 1(5), pp. 13–23. Available at: https://doi.org/https://doi.org/10.31102/attamru.2024.5.1.13-23.

Syamsul, E.S., Andani, F. and Soemarie, Y.B. (2016) 'Analgesic Activity Study of Ethanolic Extract of Callicarpa longifolia Lamk. in Mice', *Traditional Medicine Journal*, 21(2), pp. 99–103. Available at: https://doi.org/https://doi.org/10.22146/tradmedj.12824.

Tjay, T.H. and Rahardja, K. (2007) *Obat-Obat Penting: Khasiat, Penggunaan, dan Efek Sampingnya*. 7th ed. Jakarta: Elex Media Komputindo.

Tulandi, S., Paat, V. and Tiwow, G. (2022) 'Uji aktivitas analgesik ekstrak etanol daun songgolangit (Tridax procumbens L.) terhadap tikus putih (Rattus norvegicus)', *Biofarmasetikal Tropis*, 5(1), p. 66.

Wardani, I.G.A.A.K. *et al.* (2021) 'Efektivitas Analgesik Ekstrak Etil Asetat Daun Kersen (Muntingia calabura L.) Pada Mencit Putih (Mus musculas) Dengan Metode Rangsangan Panas (Hot Plate Method)', *Jurnal Ilmiah Medicamento*, 7(1), pp. 8–12. Available at:

https://doi.org/10.36733/medicamento.v7i1.
1385.