AUCTORES
Globalize your Research

Journal of Pharmaceutics and Pharmacology Research

Donatus O. Anele *

Open Access

Research Article

Assessment of the Analgesic Potential of *Medicago sativa* Leaf Extract in a Rodent Model of Acetic Acid-Induced Writhing.

Donatus O. Anele[1*], Franklin E. Igwe[2], Michael C. Orih[3], Dike Onwubiko[3] and Uwa N. Onwuchekwa[4]

[1]Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Gregory University, Uturu, Abia State, Nigeria.

[2]Department of Anatomical Pathology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

[3]Department of Chemical Pathology, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

[4]Department of Internal Medicine, College of Medicine and Health Sciences, Abia State University, Uturu, Nigeria.

***Corresponding Author:** Donatus O. Anele, Dean, Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Gregory University, Uturu, Abia State, Nigeria.

Received date: August 01, 2026; **Accepted date:** August 13, 2026; **Published date:** August 24, 2026

Citation: Donatus O. Anele, Victor Okoroukwu, Ezekwesiri Ekeke, Franklin E. Igwe, Michael C. Orih, et al., (2026), Assessment of the Analgesic Potential of *Medicago sativa* Leaf Extract in a Rodent Model of Acetic Acid-Induced Writhing, *J. Pharmaceutics and Pharmacology Research*, 9(1); **DOI:** 10.31579/2693-7247/249

Copyright: © 2026, Donatus O. Anele. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract

Background: This research investigates the analgesic potential of *Medicago sativa* (Alfalfa) leaf extract in a rodent model of acetic acid-induced writhing. *Medicago sativa*, known for its medicinal properties, has been traditionally used to alleviate pain.

Methods: Fresh leaves of *Medicago sativa* were collected, identified, and authenticated. The leaves were air-dried, pulverized, and extracted using 80% ethanol. The resulting extract was concentrated, yielding a dry extract, which was reconstituted in distilled water to achieve desired doses. Thirty adult Wistar rats were divided into five groups and pre-treated with normal saline, *Medicago sativa* leaf extract (at doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg), or acetylsalicylic acid (ASA) at 150 mg/kg. After 30 minutes, rats were subjected to acetic acid-induced writhing, and the number of abdominal constrictions was recorded for 30 minutes.

Results: The ethanol extract of *Medicago sativa* leaf demonstrated significant analgesic activity at doses of 200 mg/kg (48% inhibition), 400 mg/kg (76% inhibition), and 600 mg/kg (91% inhibition). Acetylsalicylic acid (ASA) at 150 mg/kg also exhibited significant pain inhibition (97%).

Conclusion: *Medicago sativa* leaf extract possesses analgesic potential, as evidenced by its significant reduction in acetic acid-induced writhing in Wistar rats. The findings suggest a promising avenue for further exploration of *Medicago sativa* as a natural analgesic agent.

Keywords: *Medicago sativa*, analgesic potential, rodent model, acetic acid-induced writhing, Wistar rats, ethanol leaf extract, pain inhibition

Introduction

Pain management is a critical aspect of healthcare, and researchers are continually exploring novel compounds and therapeutic strategies to alleviate pain effectively. One such avenue of investigation involves the exploration of natural products with potential analgesic properties. *Medicago sativa*, commonly known as alfalfa, has a rich history of traditional medicinal use and contains various bioactive compounds. This study aims to assess the analgesic potential of *Medicago sativa* leaf extract in a rodent model of acetic acid-induced writhing, utilizing Wistar rats.

Medicago sativa, a member of the Fabaceae family, has been traditionally employed in various cultures for its purported anti-inflammatory and analgesic properties [1]. The plant contains a diverse array of bioactive

constituents, including alkaloids, flavonoids, saponins, and terpenoids, which have demonstrated pharmacological activities in previous studies [2,3].

Acetic acid-induced writhing in rodents, particularly Wistar rats, is a well-established model for studying visceral pain and assessing potential analgesic agents. The administration of acetic acid induces abdominal contractions and writhing responses, serving as a reliable measure of nociception [4]. The use of this model allows researchers to evaluate the efficacy of analgesic compounds in mitigating pain-associated behaviors.

Previous studies have reported the analgesic effects of various plant extracts, emphasizing the potential of natural products as alternatives to conventional analgesic agents. *Medicago sativa*, with its diverse phytochemical composition, presents a promising candidate for further exploration in the context of pain management [5-7].

The rationale behind this research lies in the need for safer and more effective analgesic agents, particularly those derived from natural sources. Understanding the analgesic potential of *Medicago sativa* leaf extract in a well-established rodent model can contribute valuable insights to the development of alternative pain management strategies.

Materials and Methods

Plant Collection and Identification

Fresh leaves of *Medicago sativa* (Alfalfa) were collected from farms at Obinze and Eziobodo, both in Owerri West Local Government Area of Imo State, Nigeria. The plant was identified and authenticated by Mr. Ibe Ndukwe, a taxonomist in the Department of Forestry, College of Environmental Sciences, Michael Okpara University of Agriculture, Umudike, Abia State and a sample specimen MOUAU/ZEB/21/009 was deposited in the University herbarium for reference.

Extraction of Plant Materials

The leaves were sliced into pieces, air-dried at room temperature and pulverized into powder using a Warring commercial blender. Eight hundred grams (800 g) of the coarse powder of *M. sativa* leaves were weighed by a sensitive digital weighing balance. The powder was soaked in a flask containing 80% ethanol (2.5 L w/v) and then placed on a shaker with occasional shaking for 48 hours at room temperature. The mixture formed was filtered using Whatman (No.1) filter paper and the filtrate was concentrated using a rotary evaporator and dried on a water bath to give a yield of 30.45 g (4 % w/w) dry extract with a greenish colour. It was preserved in a refrigerator at 4℃ for further analysis. The extract was later reconstituted in distilled water to give desired doses of 200 mg/kg, 400 mg/kg, and 600 mg/kg body weight. The percentage yield was calculated using the formula of Ezirim *et al*. [8] as stated below:

$$\% \text{ yield} = \frac{\textbf{Weight(g) of residue (dry extract)}}{\textbf{Weight(g) of powdered material grinded}} \times \frac{\mathbf{100}}{\mathbf{1}}$$

Experimental Design

Thirty (30) Adult Wistar rats (200 – 250 g) of either sex were used in this study. The animals were sourced from Department of Zoology, Faculty of Biological Sciences, University of Nigeria, Nsukka. They were kept in the animal house of the Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, Abia State University, Uturu, Abia State. The animals were acclimatized for 14 days prior to the experiment. They were fed with standard diet (Ladokun feeds, Ibadan) and had access to water *ad libitum*. They were maintained under standard conditions of humidity, temperature and 12 hours light and 12 hours darkness cycle. The animals were used in accordance with the National Institute of Health guide for the care and use of Laboratory Animals [9].

The animals were divided into five groups of six each and pre-treated as follows: Group 1, which serves as control, received 0.2 mL of normal saline. Group 2, 3 and 4 received 200 mg/kg, 400 mg/kg and 600 mg/kg body weight of the *M. sativa* leaf extract, respectively, while group 5 received Acetylsalicylic acid (ASA) 150 mg/kg body weight. All the treatments were administered intraperitoneally (I.P). Thirty (30) minutes after pre-treatment, each rat was given 0.7% of an aqueous solution of acetic acid (20 ml/kg). The rats were then placed in transparent Perspex observation boxes and the number of abdominal constrictions were counted for 30 minutes after treatment. The percentage inhibitions of constrictions for the extract and ASA groups were calculated as:

$$\% \text{ inhibition} = \frac{\text{Control mean} - \text{test mean}}{\text{Control mean}}\ x\ 100$$

Results

The three doses used in the ethanol extract showed significant ($p<0.05$) activity, and the range was 48%, 76%, 91 % respectively (Table 1). The oral administration of acetylsalicylic acid (aspirin), the standard drug showed a significant ($p<0.05$) reduction in writhing and gave pain inhibitions of 97% comparable to the extract (Table 1 and Fig.1).

Table 1: The effect of ethanol leaf extract of *M. sativa* on acetic acid- induced writhing in rats.

Drug	Dose (mg/kg)	Writhes	% Inhibition
Control	20 mL/kg	37.50±2.53	-
M. sativa	200	19.50±0.43	48a
	400	8.83± 0.48	76b
	600	3.50± 0.43	91b
Aspirin	150	1.00± 0.52	97 b

One-way ANOVA + Dunnett's post hoc test (n=6). [a] $P<0.05$; [b] $P<0.01$ compared to control.

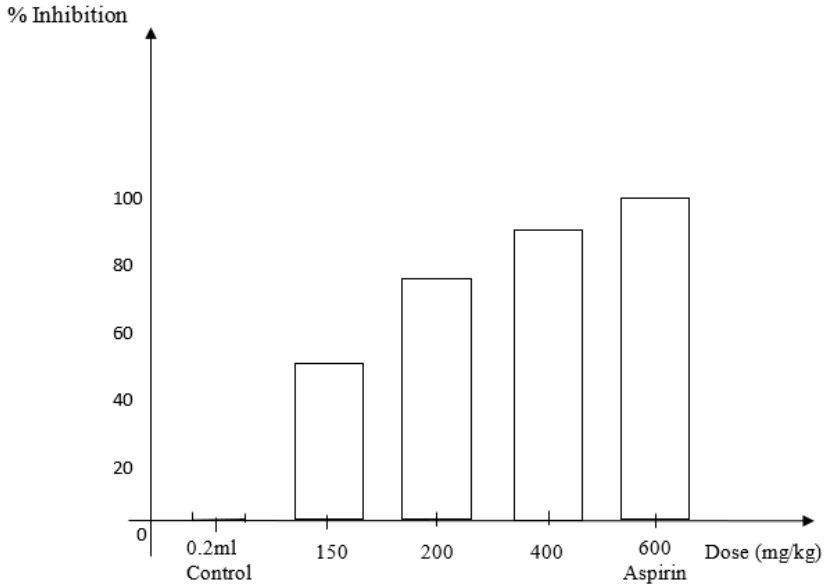

Figure 1: Bar Chart of Ethanol Leaf extract of *M. sativa* on Acetic Acid – induced writhing.

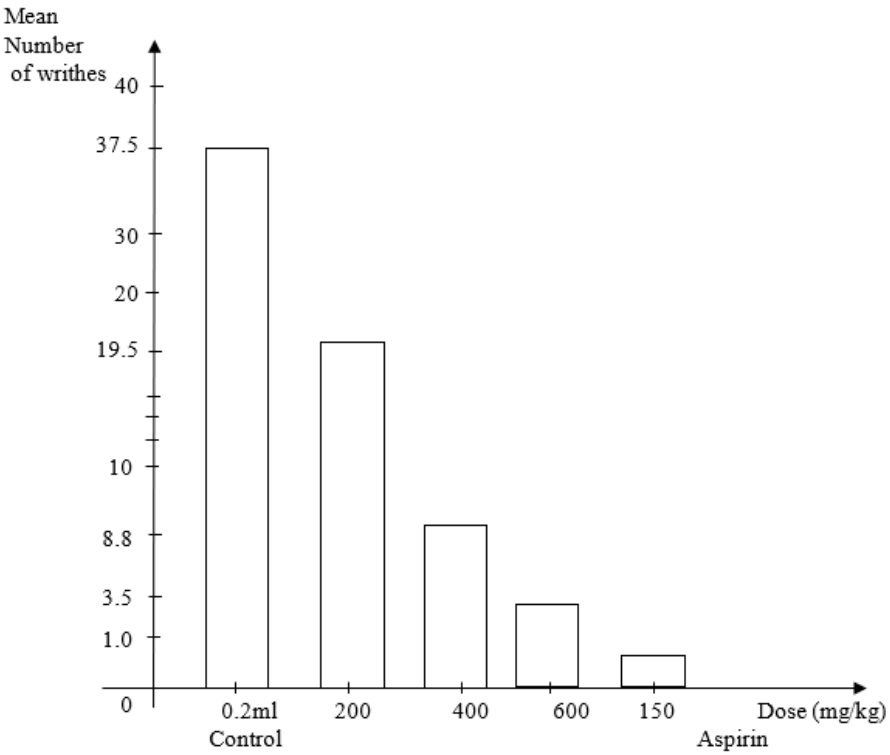

Figure 2: Oral Acute Toxicity test of *M. sativa* ethanol leaf Extract.

Discussion

Pain sensation is known as one of the reasons why people ask for medical help, and therefore drugs for pains were commonly approved to relieve pain [10,11]. The use of analgesics such as non-steroidal anti-inflammatory drugs (NSAIDs), Aspirin, opiates, as pain killers/relievers had not been successful in all cases, as a result of adverse effects, such as liver damage [12]. The strategy now is to search for clinically new and useful analgesics with negligible or no side effects. Analgesic activity of *M. sativa* was determined by acetic acid-induced writhing in rats. In this test, an intraperitoneal injection of acetic acid triggers the release of several inflammatory mediators such as prostaglandins, bradykinins, histamine, serotonin, cyclooxygenase (COX), and pro-inflammatory cytokines (IL-1, IL-6, IL-8, and TNF-α). These mediators enter the dorsal horn of CNS and stimulate primary afferent nociceptors, and initiated pain response [13,14]. The results of the present study revealed that *M. sativa* ethanol leaf extract at doses employed exhibited analgesic effect against chemically induced pain (writhing) by acetic acid [15].

The results showed that the extract had peripheral analgesic properties which suggested that the action might be directed via opposition of receptors in local peritoneal nerves [16]. Irrespective of whether the model evaluates peripheral analgesic action only or non-specific, the results validated the usefulness of this plant in Nigeria, as an analgesic agent. The injection of acetic acid is reported to induce prostaglandins and other cycloxygenase mediators [17,18]. This suggested that the ethanol extract of *M. sativa* acted by antagonizing cycloxygenase actions said to facilitate prostaglandins production through arachidonic acid [18]. The analgesic effects produced by the experimental plant (*M. sativa* leaf) significantly exhibited analgesic activity; it inhibited acid induced writhing response in the experimental animals. This pain mechanism, believed to involve local peritoneal receptors, [10,11] was caused by peritoneal fluid concentration of PG-E2 and PG-F2∝ [19]. This method is reliable and also afforded rapid evaluation of peripheral type of analgesic action and the animals reacted in a characteristic stretching manner which was referred to as writhing. Acetic acid induced writhes is a sensitive procedure in detecting analgesic effect of medicinal agents [10]. *Medicago sativa* extract was found to inhibit acetic acid-induced writhing response in the experimental animals. The abnormal constriction was related to the sensitization of pain receptors by prostaglandins. It was possible that the analgesic effect of the extract might be due to the inhibition of prostaglandin synthesis.

Our earlier study on the phytochemical composition of *M. sativa* revealed the presence of alkaloids, flavonoids, saponins and also indicated a higher level of polyphenols content (flavonoids, phenol, and tannins) in the *M. sativa* [20]. The analgesic activity of *M. sativa* might be due to the presence of alkaloids, flavonoids, phenols, and tannins since previous scientific investigations reported that these bioactive phytoconstituents have significant analgesic activity [21-24].

Earlier studies have reported the analgesic activity of some plant extracts against acetic acid induced writhing. For example, Chy *et al*. [25] reported the analgesic activity of *Piper sylvaticum leaves,* Hasan *et al*., [26] investigated the analgesic activity of ethanolic decoction of *Commelina benghalensis* roots. *Anacardium occidentale* extract has also been reported to possess analgesic activity [27]. In a related study, Boussouf et al. [24] elucidated the analgesic activity of Mentha rotundifolia L. leaves. Similarly, Morteza-Semnani et al. [23] revealed the analgesic properties of Glaucium paucilobum. The findings of the current study align with this growing body of research, reinforcing the idea that plant extracts, such as *M. sativa*, could play a significant role in the development of novel analgesic agents.

Conclusion

The results of the current study suggest that the ethanol leaf extract of *Medicago sativa* possesses significant analgesic potential in a rodent model of acetic acid-induced writhing comparable to the effects of the standard drug acetylsalicylic acid. These findings align with existing literature on the analgesic properties of plant extracts, emphasizing the importance of further research to elucidate the specific mechanisms responsible for the observed effects. The study contributes valuable insights into the potential therapeutic use of *Medicago sativa* in managing pain, opening avenues for future investigations and the development of novel analgesic agents.

References

1. Collier, H. O., Dinneen, L. C., Johnson, C. A., & Schneider, C. (2018). The abdominal constriction response and its suppression by analgesic drugs in the mouse. British Journal of Pharmacology, 32(2), 295–310.
2. Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2014). The biopsychosocial approach to chronic pain: scientific advances and future directions. Psychological Bulletin, 140(3), 807–837.
3. Lal, R., Sukbong, H., & Suthakaran, R. (2018). Opioid-induced bowel dysfunction. Journal of Neurogastroenterology and Motility, 24(4), 554–562.
4. Ong, W. Y., Stohler, C. S., & Herr, D. R. (2018). Role of the prefrontal cortex in pain processing. Molecular Neurobiology, 55(9), 7769–7785.
5. Singh, J., Dubey, R., & Tripathi, P. (2017). Anti-inflammatory activity of *Medicago sativa* Linn. (Alfalfa) in experimental animal. Asian Pacific Journal of Tropical Medicine, 10(10), 976–981.
6. Srivastava, S., Sharma, A., Rana, A. C., & Sharma, A. (2019). Alfalfa (*Medicago sativa* Linn.): A potential herbal source for health and nutrition. Journal of Food Science and Technology, 56(10), 4276–4289.
7. Trescot, A. M., Datta, S., Lee, M., & Hansen, H. (2018). Opioid pharmacology. Pain Physician, 21(2S), S133–S146.
8. Ezirim, E.O., Uche, C.L., Abali, I.O., Iwuoha, C.E., Chika-Igwenyi, N.M., Onyeaghala, C.A., Orji, S.F., Ugwu, C.N., Ugwu, N.I., Igwenyi, C. and Airaodion, A.I. (2022). Therapeutic potential of *Parkia biglobosa* seed against potassium bromate-induced testicular toxicity. *International Journal of Research and Reports in Gynaecology* 5(3): 78-89.
9. National Research Council. Guide for the Care and Use of Laboratory Animals, 8th ed. The National Academies Press: Washington, DC, USA, 2011
10. Akuodor, G.C., Idris-Usman, M., Ugwu, T.C., Akpan, J.L., Irogbeyi, L.A., Iwuanyanwu, T.C. and Osunkwo U.A. (2010). Ethanolic leaf extract of *Verbena hastate* produces antidiarrhoeal and gastrointestinal slowing effects in albino rats. *Journal of Medicinal Plants Research*, 4: 1624–1627.
11. Mota, M.A., Landim, J.S., Targino, T.S., Silva, S.F., Pereiva, M.R (2015). Evaluation of the anti-inflammatory and analgesic effects of green tea (*Camellia sinensis*) in rats. *Acta. Cir. Bras.*, vol. 30 (4): 242-246.
12. Zulfiker, A.H.M., Rahman, M.M., Hossain, M.K., Hamid, K., Mazumder, M.E.H., Rana, M.S. (2010). *In vivo* analgesic activity of ethanolic extracts of two medicinal plants – *Scoparia dulcis* L. and *Ficus racemosa* Linn. *Biology & Medicine.*2(2): 42- 48.
13. Ikeda Y, Ueno A, Naraba H, Oh-ishi S. Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. Life Sci 2021; 69:2911–2919.
14. Radu BM, Bramanti P, Osculati F, Flonta M-L, Radu M, Bertini G. Neurovascular unit in chronic pain. Mediators Inflamm 2023; 2013:1–18.
15. Sha'a, K.K., Oguche, S., Watila, I.M. & Ikpa, T.F. (2011). *In vitro* antimalarial activity of the extracts of *Vernonia*

amygdalina commonly used in traditional medicine in Nigeria. *Science World Journal*, 6 (2):5-9.

16. Mbiantcha, M., Kamanyi, A., Teponno, R. B, Tapondjou, A.L., Watcho, P. & Nguelefack T. (2010). Analgesic and Anti-Inflammatory Properties of Extracts from the Bulbils of *Dioscorea bulbifera* L.*var sativa* (Dioscoreaceae) in Rats and Rats. *Evidence Based Complementary Alternative Medicine.* (in process).
17. Divya, T.S., Latha, P.G., Usha, K., Anuja,G.I., Suja, S.R., Shyamal, S., Shine, V.J., Sini, S.,Shikha, P. & Rajasekharan, S. (2009). Anti-inflammatory, analgesic and anti-lipid peroxidative properties of *Wattakaka volubilis* (Linn.f.) Stapf. *Natural Product Radiance*, 8(2): 137-141.
18. Nkeh-Chungag, N.B., Bekwa, P.C.M., Ndebia, J.E., Kayo, M., Mbafor, T.J. & Iputo, E.J. (2010). Analgesic and anti-inflammatory properties of *Oxyanthus unilocularis*. *Journal of Medicinal Plants Research*, 4(10): 932-939.
19. Tarkang, P.A., Agbor, G.A., Armelle, T.D., Yamthe, T.L.R., David, K., Ngadena, Y.S.M (2012). Acute and chronic toxicity studies of the aqueous and ethanol leaf extracts of Carica papaya Linn in Wistar rats. *J Nat Prod Plant Resour*. 2(5): 617-27.
20. Anele et al.,
21. Küpeli E, Yesilada E. Flavonoids with anti-inflammatory and antinociceptive activity from Cistus laurifolius L. leaves through bioassay-guided procedures. J Ethnopharmacol 2007; 112:524–530.
22. Starec M. Evaluation of the analgesic effect of RG-tannin using the “hot plate” and “tail flick” method in mice (in Czech). Ces Farm 2018; 37:319–321.
23. Morteza-Semnani K, Mahmoudi M, Heidar MR. Analgesic activity of the methanolic extract and total alkaloids of Glaucium paucilobum. Methods Find Exp Clin Pharmacol 2016; 28:151–156.
24. Boussouf L, Boutennoune H, Kebieche M, Adjeroud N, Al-Qaoud K, Madani K. Anti-inflammatory, analgesic and antioxidant effects of phenolic compound from Algerian Mentha rotundifolia L. leaves on experimental animals. S Afr J Bot 2017; 113:77–83.
25. Chy, M., Chakrabarty, N., Roy, A., Paul, A., Emu, K., Dutta, T., Dutta, E., Ferdous, I., Das, R., Hasan, M. & Tasnim, S. (2019). Antibacterial, anthelmintic, and analgesic activities of Piper sylvaticum (Roxb.) leaves and in silico molecular docking and PASS prediction studies of its isolated compounds. *Journal of Complementary and Integrative Medicine*, *16*(4), 20180176.
26. Hasan SMR, Hossain MM, Akter R, Jamila M, Mazumder MEH, Alam MA *et al*. Anal Parts Analgesic Activity of the Different Fractions of the Aerial Parts of *Commelina benghalensis* Linn. International Journal of Pharmacology, 2010; 6(1):63-67.
27. Aderiye BI, David OM, Atere VA, 2015. Administration of cashew extracts in the treatment of some infections and diseases. Adv Med Plant Res, 3(3): 75-86.

This work is licensed under Creative Commons Attribution 4.0 License

To Submit Your Article Click Here: **Submit Manuscript**

DOI: 10.31579/2693-7247/249

Ready to submit your research? Choose Auctores and benefit from:

- fast, convenient online submission
- rigorous peer review by experienced research in your field
- rapid publication on acceptance
- authors retain copyrights
- unique DOI for all articles
- immediate, unrestricted online access

At Auctores, research is always in progress.

Learn more https://auctoresonline.org/journals/pharmaceutics-and-pharmacology-research