Journal of Chemical Health Risks

www.jchr.org

JCHR (2026) 16(2), 3431-3442 | ISSN:2251-6727

Phytochemical Profile, Acute Toxicity, and Antinociceptive Potential of Methanolic Extract of Aerva Sanguinolenta (L.) Blume in Swiss Albino Mice

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(Received: 16 February 2026 Revised: 25 March 2026 Accepted: 10 April 2026)

KEYWORDS

Aerva sanguinolenta; Antinociceptive activity; Medicinal plants; Pain models; Phytochemicals; Analgesic activity

ABSTRACT:

Introduction: Pain is a complex physiological and pathological condition that significantly impairs quality of life. Although conventional analgesics such as NSAIDs and opioids are widely used, their long-term use is associated with adverse effects, prompting interest in medicinal plants as safer alternatives.

Objective: This study aimed to evaluate the antinociceptive potential of the methanolic extract of *Aerva sanguinolenta* (MEAS) and explore its possible mechanisms of action.

Methods: The crude methanolic extract of *A. sanguinolenta* was prepared by maceration and subjected to preliminary phytochemical screening. Acute toxicity was assessed following OECD guidelines. Antinociceptive activity was evaluated using tail immersion, acetic acid-induced writhing, formalin-induced nociception, and glutamate-induced nociception models in Swiss albino mice at doses of 100, 200, and 400 mg/kg BW. Morphine and diclofenac sodium were used as standard drugs. Data were analyzed using one-way ANOVA followed by post hoc tests.

Results: Phytochemical analysis revealed the presence of flavonoids, phenolics, glycosides, alkaloids, terpenoids, and saponins. The extract was found to be safe with no observed toxicity. MEAS produced significant, dose-dependent antinociceptive effects across all models. In the tail immersion test, it increased latency time, indicating central analgesic activity. In the acetic acid-induced writhing test, MEAS significantly reduced writhing responses, demonstrating peripheral analgesic effects. In the formalin test, the extract inhibited both neurogenic and inflammatory phases, with greater efficacy in the late phase. Additionally, MEAS significantly reduced glutamate-induced nociception, suggesting involvement of glutamatergic pathways.

Conclusion: The findings demonstrate that *Aerva sanguinolenta* possesses significant antinociceptive activity mediated through both central and peripheral mechanisms. These effects are likely attributed to its bioactive phytoconstituents, particularly flavonoids and phenolics. The study supports the traditional use of this plant in pain management and highlights its potential as a source of novel analgesic agents. Further studies are warranted to isolate active compounds and elucidate the precise mechanisms of action.

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JCHR (2026) 16(2), 3431-3442 | ISSN:2251-6727

Introduction

Pain is a multidimensional phenomenon encompassing both sensory and emotional components, arising from actual or potential tissue injury and serving as a crucial biological warning system that protects the body from further harm [1]. It can be categorized according to its duration into acute, subchronic, and chronic forms [2], as well as by its underlying origin into neuropathic and inflammatory types [1].

Acute pain typically occurs rapidly in response to injury and plays a vital role in preventing further tissue damage and facilitating the healing processes [2]. In contrast, chronic pain persists beyond the normal healing period, often exceeding several days, and represents a significant global health burden. It adversely affects individuals across multiple dimensions, including physical functioning, emotional well-being, economic productivity, and social interactions [3,4]. Regardless of the type, effective pain management is generally required, most commonly through the administration of analgesic medications.

Among these, nonsteroidal anti-inflammatory drugs (NSAIDs) are extensively utilized for the management of mild to severe pain conditions [5]. Their pharmacological action primarily involves the inhibition of cyclooxygenase enzymes (COX-1 and COX-2) at both peripheral and central levels, thereby suppressing the biosynthesis of key inflammatory mediators such as prostaglandins, thromboxanes, and leukotrienes [6]. Despite their widespread use, prolonged NSAID therapy is frequently associated with adverse effects, including gastrointestinal disturbances, abdominal discomfort, dermatological reactions, and renal complications [6]. Furthermore, their therapeutic limitations often necessitate the use of opioid analgesics, which carry a higher risk of serious side effects and dependence. Consequently, there is a pressing need to identify safer and more effective alternative analgesic agents to improve pain management strategies [7].

Medicinal plants have long served as valuable sources of therapeutic agents for the treatment of various diseases [8]. The pharmacological activities attributed to these plants are largely due to the presence of diverse bioactive constituents, highlighting their potential as reservoirs of novel drug candidates [9]. Such natural compounds continue to play an important role in the discovery and development of new analgesic agents [10]. Notably, plant bark has been widely used in traditional medicine systems for centuries. It is known to contain a variety of active phytochemicals, including tannins, salicylates, flavonoids, and alkaloids, which contribute significantly to its analgesic properties [11,12].

Aerva sanguinolenta (L.) Blume (family: Amaranthaceae) is a medicinal plant widely found in tropical and subtropical regions, including Bangladesh. It has traditionally been used to treat various ailments, including pain, inflammation, and infections [13,14]. Previous pharmacological research has shown that this plant exhibits antioxidant, antimicrobial, and anticancer activities, likely due to its rich phytochemical content [14,15]. However, despite its traditional use, scientific evidence supporting its antinociceptive potential remains limited.

Therefore, the present study aimed to evaluate the antinociceptive activity of the methanolic extract of *A. sanguinolenta* using established experimental models, including tail immersion, acetic acid-induced writhing, formalin-induced nociception, and glutamate-induced nociception in mice. Additionally, preliminary phytochemical screening and acute toxicity tests were conducted to assess safety and identify the bioactive compounds responsible for the observed effects.

Materials and Methods

Plant Material and Extraction

Fresh aerial parts of *Aerva sanguinolenta* were collected from the Rajshahi University campus, Bangladesh (24°22'26"N 88°36'04"E) at an altitude of approximately 23 meters above sea level in November-December. The plant was taxonomically authenticated at the Bangladesh National Herbarium, where a voucher specimen (Accession No. 46770) was deposited. The collected material was shade-dried, pulverized into coarse powder, and subjected to maceration in methanol (1:5 w/v) for 14 days with occasional agitation. The extract was then filtered and concentrated under reduced pressure using a

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rotary evaporator to obtain a crude methanolic extract (MEAS), yielding 5.495% (w/w).

Experimental Animals

Male Swiss albino mice (11–12 weeks old; 30–35 g) were obtained from the animal facility of the Department of Pharmacy, University of Rajshahi. Animals were maintained under standard laboratory conditions (12 h light/dark cycle, 27 ± 2°C, 55 ± 10% humidity) with free access to food and water. All experimental procedures were conducted in accordance with institutional ethical guidelines approved by IBSc, Rajshahi University (License no: 72 (23)/320/IAMEBBC/IBSc).

Drugs and Treatment Protocol

Mice were randomly allocated into four experimental models (n = 25), and each model was then divided into five groups (n = 5). Group I received 10 mL/kg BW of distilled water (vehicle) in the four models, whereas groups II– IV received 100, 200, and 400 mg/kg BW of MEAS, respectively. Group V was given 5 mg/kg BW of Morphine (standard drug) in tail immersion test and 10 mg/kg BW of Diclofenac Sodium (standard drug) in the acetic acid, formalin and glutamate tests. To prepare each dosage of medicine and MEAS, deionized water was utilized.

Phytochemical Screening

The crude methanolic extract of *A. sanguinolenta* (MEAS) contained saponins, flavonoids, terpenoids, glycosides, phenolics, and alkaloids, as determined by accepted techniques [15,16].

Acute toxicity study

Acute oral toxicity was evaluated according to OECD guideline 425 [17]. Mice were administered MEAS at doses ranging from 100 to 2000 mg/kg BW and monitored for 24 hours for mortality and behavioral changes, including locomotor activity, grooming, convulsions, and autonomic responses.

Evaluation of antinociceptive activity

Tail Immersion Test

Central analgesic activity was assessed using the tail immersion method as described previously [18]. The distal portion (1–2 cm) of the mouse tail was immersed in water maintained at 54 ± 0.5 °C, and the latency to tail withdrawal was recorded with a cut-off time of 20 seconds to prevent tissue damage. Measurements were taken at 0, 30, 60, and 90 minutes after treatment. The percentage of maximum possible effect (%MPE) was calculated using the following standard formula.

% MPE = [(Post-drug latency) − (Pre-drug latency) / (Cut off time) − (Pre-drug latency)] × 100.

Acetic Acid-Induced Nociception

Peripheral nociception was evaluated by intraperitoneal injection of 0.6% acetic acid [18]. Fifteen minutes after treatment, mice were observed for 30 minutes, and the number of writhing responses (abdominal constrictions) was recorded. The percentage inhibition of writhing was calculated relative to the control group.

Formalin-Induced Nociception

The protocol described by Santos & Calixto and Santos *et al*. [19,20] was followed when performing the formalin test. Mice received a subplantar injection of 2.5% formalin (20 µL) into the hind paw. The duration of paw licking was recorded during the early (0–5 min) and late (15–30 min) phases. Test and standard drugs were administered 60 minutes before formalin injection. Antinociceptive activity was expressed as percentage inhibition of licking time.

Glutamate-Induced Nociception

The glutamate-induced nociception model was performed following the method described by Beirith *et al*. [21] with minor modifications. Mice were pretreated with MEAS (100, 200, and 400 mg/kg BW, p.o.) 30 minutes before the experiment, while the standard group received diclofenac sodium (10 mg/kg BW, i.p.) 15 minutes before testing. The control group was administered deionized water (0.1 mL/mouse, p.o.). Subsequently, glutamate solution (20 µL, 10 µmol/paw) was injected into the hind paw to induce nociception. After injection, animals were individually observed for 15 minutes, and nociceptive response was assessed by measuring the duration of paw licking.

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JCHR (2026) 16(2), 3431-3442 | ISSN:2251-6727

Statistical analysis

The results are presented as the Mean ± SEM of five mice. Data were analyzed using 1-way ANOVA analysis of variance (ANOVA) followed by Dunnett's post hoc tests using Graph Prism 6 Software (Graph Pad Software, Inc., CA, USA). The results were considered significant at $p<0.05$.

Results

Phytochemical Screening

Preliminary phytochemical analysis of the crude methanolic extract of *Aerva sanguinolenta* (MEAS) revealed the presence of several bioactive secondary metabolites (**Table 1**). The extract showed a high abundance (+++) of flavonoids and phenolic compounds, moderate amounts (++) of glycosides, and mild presence (+) of saponins, terpenoids, and alkaloids. However, tannins and proteins were not detected in the extract.

Table 1. Qualitative tests of crude methanolic extract of *A. sanguinolenta*.

Phyto-constituents	Saponins	Tannins	Flavonoids	Terpenoid	Glycoside	Protein	Phenolics	Alkaloid
CME	+	-	+++	+	++	-	+++	+

(-) = Not present; (+) = Present in mild amount; (++) = Present in moderate amount; (+++) = Present in significant amount

Here, CME = Crude methanolic extract

Acute oral toxicity studies and dose selection

The acute oral toxicity study demonstrated that MEAS was safe at doses up to 4000 mg/kg BW, with no observed mortality or noticeable behavioral or physiological abnormalities in the treated animals during the observation period. Based on these findings, the extract can be considered non-toxic under the tested conditions. Therefore, doses of 100, 200, and 400 mg/kg BW were selected for subsequent pharmacological evaluations.

Antinociceptive Effect of MEAS in Tail Immersion Test

MEAS produced a dose- and time-dependent increase in tail withdrawal latency compared to the control group (**Table 2**; **Figure 1**). The highest effect was observed at 400 mg/kg, showing a latency of 4.79 ± 0.06 s at 90 minutes, followed by 200 mg/kg BW and 100 mg/kg BW concentration. The percentage elongation also increased with dose, with MEAS (400 mg/kg BW) reaching 46.48% at 90 minutes. Significant effects were observed at various time points ($p < 0.05$ to $p < 0.0001$). Morphine (5 mg/kg BW) showed a markedly higher effect, with maximum latency of 7.76 ± 0.18 s and 163.67% elongation.

Table 2. Effect of *A. sanguinolenta* methanolic extract on tail immersion test.

Group	Dose (mg/kg BW)	Avg. time of immersion (Mean ± SEM)				% Elongation			
		0 minutes	30 minutes	60 minutes	90 minutes	0 minutes	30 minutes	60 minutes	90 minutes

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MEAS 100	100	2.22 ± 0.05	2.87 ± 0.04	3.29 ± 0.04	3.48 ± 0.03 $^{\alpha}$	6.73	3.24	2.88	6.42
MEAS 200	200	2.69 ± 0.03 $^{\beta}$	3.31 ± 0.03 $^{\beta}$	3.82 ± 0.03 $^{\beta}$	3.97 ± 0.10 $^{\alpha}$	29.33	19.06	21.94	21.41
MEAS 400	400	3.06 ± 0.10 $^{\alpha}$	3.59 ± 0.13 $^{\alpha}$	4.33 ± 0.07 $^{\beta}$	4.79 ± 0.06 $^{\epsilon}$	47.12	29.14	40.29	46.48
STD	5	5.13 ± 0.06	6.04 ± 0.05 $^{\epsilon}$	7.76 ± 0.18 $^{\epsilon}$	6.56 ± 0.16	146.63	117.27	163.67	100.61
CTL	0.1 ml/mouse	2.08 ± 0.05	2.78 ± 0.06	3.21 ± 0.07	3.27 ± 0.03	-	-	-	-

Here, values are expressed as Mean ± SEM (n = 5); CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; $^{\epsilon}p < 0.0001$, $p < 0.001$, $^{\beta}p < 0.01$, $^{\alpha}p < 0.05$ compared with the control group.

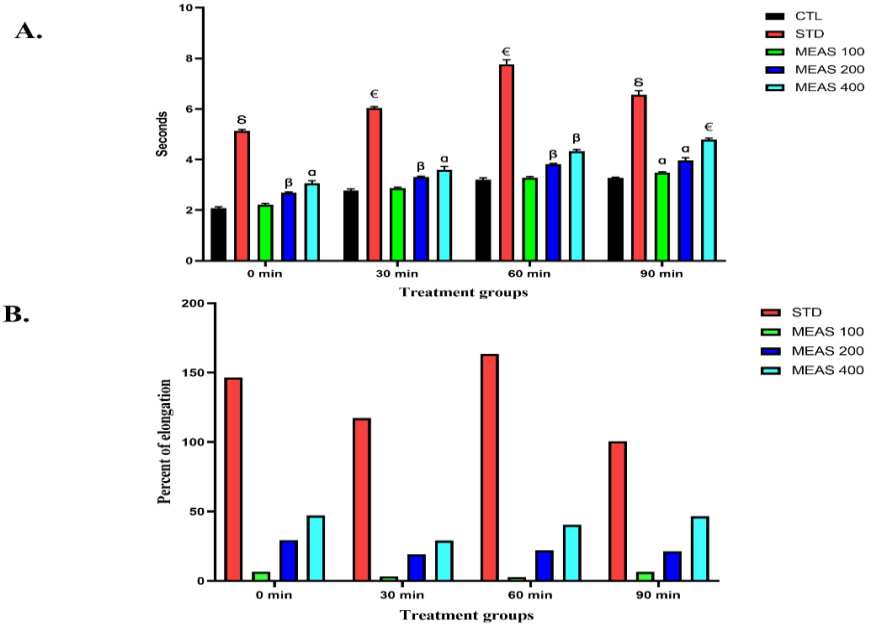

Figure 1. Antinociceptive effect of *A. sanguinolenta* methanolic extract in tail immersion test. (A) Average time of immersion; (B) Percent of elongation. All values are presented as mean ± SEM (n = 5). CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; $^{\epsilon}p < 0.0001$, $p < 0.001$, $^{\beta}p < 0.01$, $^{\alpha}p < 0.05$ compared with the control group.

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Antinociceptive Effect of MEAS in Acetic Acid–Induced Writhing Test

MEAS exhibited a significant, dose-dependent reduction in acetic acid-induced writhing in mice (**Table 3**; **Figure 2**). The control group showed a mean writhing count of 23.2 ± 0.37. Treatment with MEAS at doses of 100, 200, and 400 mg/kg BW reduced the number of writhes to 17.4 ± 1.03, 12.2 ± 0.37, and 9.6 ± 0.51, respectively. These reductions correspond to inhibition percentages of 25.00%, 47.41%, and 58.62%, respectively. The effects were statistically significant at all doses, with higher significance observed at 200 mg/kg BW (****p < 0.0001) and 400 mg/kg BW (***p < 0.001). The standard drug, diclofenac sodium (10 mg/kg BW), produced a more pronounced inhibition (75.00%), with a writhing count of 5.8 ± 0.49 (***p < 0.001).

Table 3. Effect of crude methanolic extract of *A. sanguinolenta* on acetic acid-induced abdominal writhing test.

Treatment	Dose (mg/kg BW)	Mean ± SEM	% Inhibition
MEAS 100	100	17.4 ± 1.03*	25.00
MEAS 200	200	12.2 ± 0.37****	47.41
MEAS 400	400	9.6 ± 0.51***	58.62
STD	10	5.8 ± 0.49***	75.00
CTL	0.1 ml/mouse	23.2 ± 0.37	0.00

Here, values are expressed as Mean ± SEM (n = 5); CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; ****p < 0.0001, ***p < 0.001, *p < 0.05 compared with the control group.

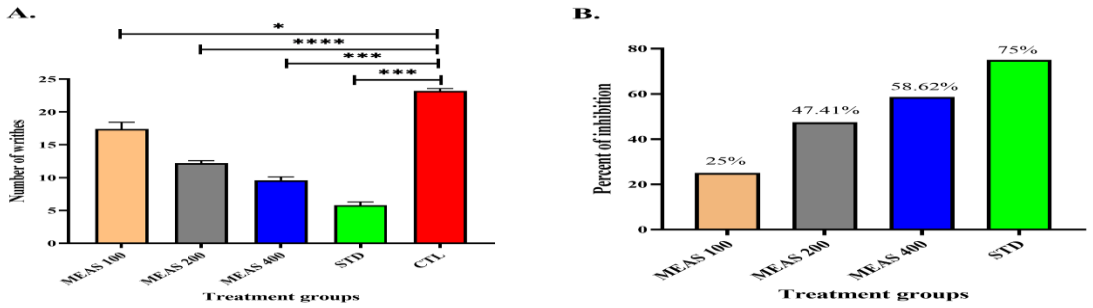

Figure 2. Antinociceptive effect of *A. sanguinolenta* methanolic extract in acetic acid-induced writhing. (A) Number of writhes; (B) Percent of inhibition. All values are presented as mean ± SEM (n = 5). CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; ****p < 0.0001, ***p < 0.001, *p < 0.05 compared with the control group.

Antinociceptive effect of MEAS in Formalin Test

In the formalin test, MEAS significantly reduced paw-licking time in both the early (neurogenic) and late (inflammatory) phases in a dose-dependent manner (**Table 4**; **Figure 3**).

In the early phase, the control group exhibited a licking time of 64.08 ± 3.14 s. MEAS treatment reduced this to 56.84 ± 2.28 s (11.30% inhibition), 39.72 ± 1.41 s (38.01% inhibition, *p < 0.05), and 29.9 ± 0.75 s (53.34% inhibition, **p < 0.01) at doses of 100, 200, and 400 mg/kg BW,

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respectively. The standard drug showed 60.02% inhibition (**p < 0.01).

In the late phase, the control group showed a licking time of 35.88 ± 1.21 s. MEAS significantly reduced this to 31.56 ± 0.81 s (12.04% inhibition), 20.76 ± 1.17 s (42.14% inhibition, **p < 0.01), and 11.96 ± 0.97 s (66.67% inhibition, ***p < 0.001) at doses of 100, 200, and 400 mg/kg BW, respectively. Diclofenac sodium exhibited 72.13% inhibition (***p < 0.001).

Notably, the highest dose of MEAS (400 mg/kg BW) demonstrated an effect comparable to the standard drug, particularly in the late phase.

Table 4. Antinociceptive effects of *A. sanguinolenta* crude extract in formalin-induced nociception.

Treatment	Dose (mg/kg BW)	Licking of the hind paw			
		Early Phase	% Inhibition	Late Phase	% Inhibition
MEAS 100	100	56.84 ± 2.28	11.3	31.56 ± 0.81	12.04
MEAS 200	200	39.72 ± 1.41*	38.01	20.76 ± 1.17**	42.14
MEAS 400	400	29.9 ± 0.75**	53.34	11.96 ± 0.97***	66.67
STD	10	25.62 ± 0.57**	60.02	10.00 ± 0.49***	72.13
CTL	0.1 ml/mouse	64.08 ± 3.14	0.00	35.88 ± 1.21	0.00

Here, values are expressed as Mean ± SEM (n = 5); CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; ***p < 0.001, **p < 0.01, *p < 0.05 compared with the control group.

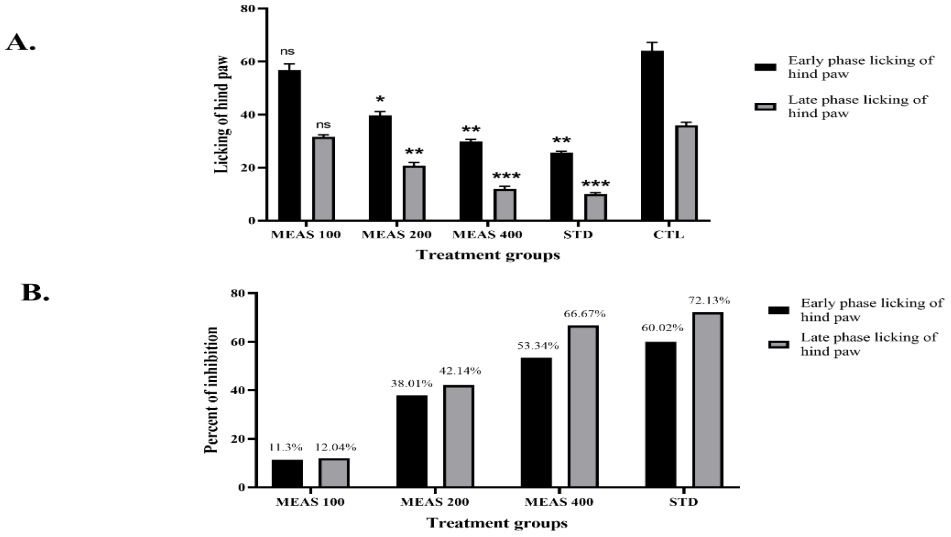

Figure 3. Antinociceptive effect of *A. sanguinolenta* methanolic extract in formalin-induced nociception. (A) Number of paw licking; (B) Percent of inhibition. All values are presented as mean ± SEM (n = 5). CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; ***p < 0.001, **p < 0.01, *p < 0.05 compared with the control group, ns = not significant.

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Antinociceptive effect of MEAS in Glutamate Test

MEAS significantly attenuated glutamate-induced nociception in mice, as evidenced by reduced licking time (**Table 5**; **Figure 4**). The control group exhibited a licking time of 34.4 ± 0.93 s. Treatment with MEAS at 100, 200, and 400 mg/kg BW reduced the licking time to 29.4 ± 1.47 s (**p < 0.01), 19.0 ± 1.05 s (***p < 0.001), and 12.6 ± 0.93 s (**p < 0.01), respectively.

These correspond to inhibition percentages of 14.53%, 44.77%, and 63.37%, respectively, indicating a clear dose-dependent effect. The standard drug, diclofenac sodium (10 mg/kg BW), produced a significant reduction (13.8 ± 1.07 s) with 59.88% inhibition (****p < 0.0001).

Interestingly, the highest dose of MEAS (400 mg/kg BW) showed a slightly higher inhibition than the standard drug, suggesting potent antinociceptive activity.

Table 5. Antinociceptive effects of *A. sanguinolenta* crude extract in glutamate-induced nociception.

Treatment	Dose (mg/kg BW)	Licking time (s)	% Inhibition
MEAS 100	100	29.4 ± 1.47**	14.53
MEAS 200	200	19 ± 1.05***	44.77
MEAS 400	400	12.6 ± 0.93**	63.37
STD	10	13.8 ± 1.07****	59.88
CTL	0.1 ml/mouse	34.4 ± 0.93	0.00

Here, values are expressed as Mean ± SEM (n = 5); CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; ****p < 0.0001, ***p < 0.001, **p < 0.01 compared with the control group.

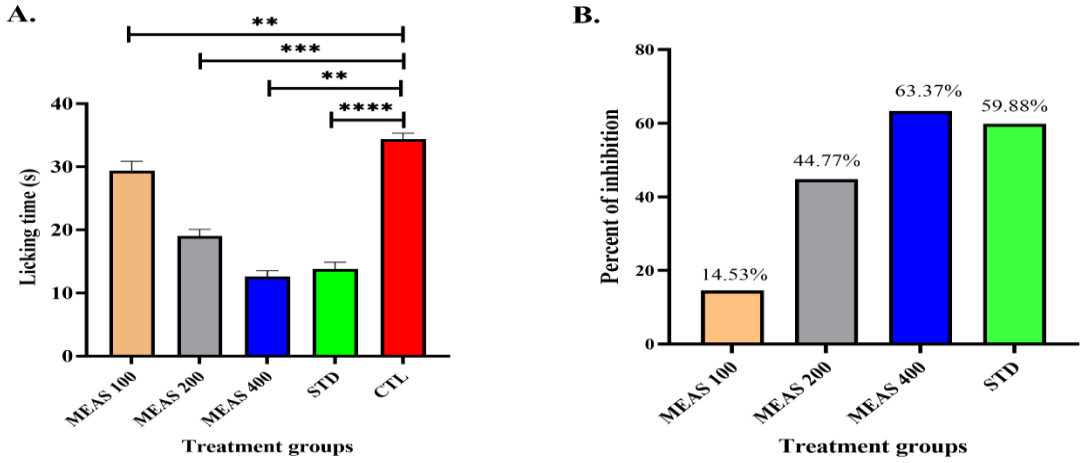

Figure 4. Antinociceptive effect of *A. sanguinolenta* methanolic extract in glutamate-induced nociception. (A) Licking time; (B) Percent of inhibition. All values are presented as mean ± SEM (n = 5). CTL = Control, STD = Standard, MEAS = Methanolic extract of *A. sanguinolenta*; ****p < 0.0001, ***p < 0.001, **p < 0.01 compared with the control group.

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Discussion

The preliminary phytochemical screening of the methanolic extract of *Aerva sanguinolenta* revealed the presence of flavonoids, phenolics, glycosides, alkaloids, terpenoids, and saponins. Among these, flavonoids and phenolic compounds were found in significant amounts, suggesting their possible contribution to the observed antinociceptive activity. These classes of compounds are well documented for their analgesic and anti-inflammatory properties, primarily through inhibition of prostaglandin synthesis, modulation of oxidative stress, and interaction with nociceptive pathways [22].

The acetic acid-induced writhing test is a widely used model for evaluating peripheral analgesic activity [23]. It is associated with the release of endogenous substances such as prostaglandins (particularly PGE_2 and $PGF_2\alpha$), bradykinin, and cytokines, which stimulate nociceptive neurons [24]. In this study, MEAS significantly reduced the number of writhes in a dose-dependent manner, indicating its peripheral antinociceptive effect. The observed inhibition suggests that the extract may interfere with the synthesis or action of these inflammatory mediators. The effectiveness of MEAS, particularly at higher doses, supports its potential role in inhibiting cyclooxygenase (COX) pathways, similar to the mechanism of diclofenac sodium.

The formalin test provides insight into both neurogenic (early phase) and inflammatory (late phase) pain mechanisms. The early phase is attributed to direct activation of nociceptors, while the late phase is associated with inflammatory processes and central sensitization [25]. In the present study, MEAS significantly reduced paw-licking time in both phases, although the effect was more pronounced in the late phase. This suggests that the extract possesses both central and peripheral analgesic properties, with stronger activity against inflammatory pain. The comparable effect of the higher dose (400 mg/kg BW) with diclofenac sodium in the late phase further reinforces its anti-inflammatory potential. The moderate inhibition observed in the early phase may indicate partial involvement of central mechanisms, possibly through modulation of neurotransmitters or ion channels involved in pain signaling.

Glutamate plays a key role as an excitatory neurotransmitter in nociceptive pathways, particularly through activation of NMDA and non-NMDA receptors [26]. Glutamate-induced nociception is associated with increased intracellular calcium levels, nitric oxide production, and activation of various signaling cascades involved in pain perception [27]. MEAS significantly reduced glutamate-induced licking behavior in a dose-dependent manner, indicating its ability to modulate glutamatergic transmission. The marked inhibition at higher doses suggests that the extract may interfere with glutamate receptors or downstream signaling pathways, thereby reducing nociceptive responses.

Taken together, the results from different nociceptive models indicate that MEAS exerts significant antinociceptive effects through multiple mechanisms, including inhibition of peripheral inflammatory mediators and modulation of central pain pathways. The dose-dependent response observed across all models further strengthens the pharmacological relevance of the extract. The presence of bioactive phytochemicals, particularly flavonoids and phenolics, likely plays a crucial role in mediating these effects.

Conclusion

The present study demonstrates that the methanolic extract of *Aerva sanguinolenta* possesses significant antinociceptive activity in various experimental models of pain. The extract exhibited dose-dependent inhibition of nociception in tail immersion, acetic acid-induced, formalin-induced, and glutamate-induced models, suggesting involvement of both peripheral and central mechanisms. The observed analgesic effects may be attributed to the presence of bioactive phytoconstituents such as flavonoids and phenolic compounds. Additionally, the extract was found to be safe in acute toxicity studies, supporting its potential therapeutic applicability. Overall, these findings provide scientific evidence supporting the traditional use of *A. sanguinolenta* in pain management and highlight its potential as a source of novel analgesic agents. However, further studies are required to isolate and

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characterize the active compounds and to elucidate the precise molecular mechanisms underlying their antinociceptive effects.

Abbreviations

MEAS, methanolic extract of *Aerva sanguinolenta*; NSAIDs, non-steroidal anti-inflammatory drugs; COX, cyclooxygenase; OECD, Organization for Economic Co-operation and Development; BW, body weight; i.p., intraperitoneal; p.o., per oral; ANOVA, analysis of variance; SEM, standard error of the mean; MPE, maximum possible effect; CTL, control; STD, standard; PGE_2, prostaglandin E_2; $PGF_2\alpha$, prostaglandin F_2 alpha; NMDA, N-methyl-D-aspartate.

Conflicts of Interest

We neither have any conflicts of interest regarding this publication nor substantial financial support that might have influenced the outcomes.

Author Contribution

Joy Sarker: conceptualization, investigation, formal analysis, data curation, writing original manuscript, correspondence; **S. M. Backther Nawaz & Most Nilufar Yeasmin:** formal analysis, software, review and editing manuscript; **F. M. Al-Amin Kaisar and Mohammed Shorfuddin Patowary:** data curation, validation, review and editing manuscript; **Khirul Islam Razu & Md. Rashedul Islam:** methodology; **Monirul Islam:** supervision, data curation, funding, review and editing manuscript.

Funding

There was no specific financing for this research project from public or private funding organization.

Ethics Approval

All methods of animal experiments were implemented following proper rules and directives of the institution, along with ethical authorization granted from the Institute of Biological Sciences (IBSc), Rajshahi University, Bangladesh (License no: 72 (23)/320/IAMEBBC/IBSc).

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