Original Article

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Methanolic Extract and its Dichloromethane Fraction of Monochoria hastata Leaves: Neuropharmacological, Antidiarrheal, Antinociceptive, and Antioxidant Effects

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Abstract:

Objective: *Monochoria hastata* (L.) Solms, an aquatic plant traditionally used in folk medicine, was investigated for its pharmacological properties using methanolic extract (ME) and dichloromethane fraction (DCMF) of the leaves.

Material and Methods: Standard assays were used to test for phytochemicals; 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) scavenging tests were used to assess the antioxidant activity. The castor oil-induced diarrhea theory was utilized to investigate antidiarrheal effects, the acetic acid-induced writhing test was employed to measure analgesic activity, and the elevated plus maze (EPM) and hole board tests were used to assess anxiolytic potential in mice (200/400 mg/kg dosages).

Results: The ME demonstrated superior DPPH radical scavenging activity (IC_{50}: 0.017 µg/mL) compared to DCMF and ascorbic acid, while DCMF exhibited better hydrogen peroxide scavenging (IC_{50}: 111.59 µg/mL). In a castor oil-induced diarrheal model, both extracts showed dose-dependent inhibition, with DCMF (400 mg/kg) achieving 76.47% inhibition. ME also displayed significant analgesic activity, reducing acetic acid-induced writhing by 56.25%, comparable to diclofenac sodium. Anxiolytic potential was confirmed using the hole board and elevated plus maze tests, where DCMF significantly increased open-arm duration and head-dipping behavior.

Conclusion: These results validate the traditional use of *M. hastata* and suggest its potential as a source of bioactive compounds for the treatment of oxidative stress, pain, diarrhoea, and anxiety-related disorders.

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Introduction

For centuries, medicinal plants have played an essential role in human health and well-being. Across cultures and generations, they have been revered not only for their nutritional value but also for their therapeutic benefits. These plants are widely appreciated for being cost-effective, efficient, and generally safe, with minimal toxicity[1]. Their rich content of pharmacologically active compounds has secured their place in traditional medicine systems across the globe [2]. Nearly 80% of the world's population uses traditional medicines, reports the World Health Organization [3].

The medicinal value of plants largely stems from the diverse secondary metabolites they produce to defend against herbivores, pathogens, and environmental stress. These compounds, such as phenols, flavonoids, and alkaloids, not only serve ecological functions but also offer significant therapeutic benefits to humans. Numerous studies have confirmed their antibacterial, antifungal, antioxidant, anti-inflammatory, anticancer, and hepatoprotective properties[4,5].

While much attention has been given to terrestrial plants, aquatic and wetland species have been comparatively neglected in phytochemical research. Yet, recent interest has begun to uncover the immense potential of aquatic plants as sources of bioactive compounds. These plants, often dismissed for their role in eutrophication, a process in which excessive nutrients lead to algal blooms and water quality degradation[6], actually possess unique metabolic adaptations. Through specialized chemical defenses shaped by their environment, aquatic plants are capable of producing a variety of pharmacologically important secondary metabolites[7].

Among these promising species is *Monochoria hastata*, an aquatic plant from the family Pontederiaceae. Known for its striking arrow-shaped leaves and vibrant purple-blue flowers, it is commonly referred to as "Arrow-leaf pondweed" in English, "Bara nukha" in Bengali, and "Nilotpalam" in Sanskrit. Traditional medicine practitioners everywhere in Asia, but especially on the Indian subcontinent, have long relied on this plant. In traditional medicine, it is used as a vegetable to cure a variety of conditions, including cuts, boils, indigestion, and liver issues[8–11].

Inspired by its ethnomedicinal significance and the growing need to explore aquatic biodiversity, the present study focuses on *M. hastata*. The research aimed to systematically evaluate the chemical composition and pharmacological activities of its methanolic leaf extract and dichloromethane fraction. Comprehensive phytochemical screening was conducted to identify key bioactive compounds, followed by assessments of various biological activities, including antioxidant, antidiarrheal, antinociceptive, and neuropharmacological effects. This integrative approach sought to validate traditional knowledge with scientific evidence and highlight the therapeutic potential of *Monochoria hastata* as a candidate for drug discovery and development.

Material and Methods

Drugs and Chemicals

The analytical grade was maintained throughout the study by using pure chemicals and reagents. We obtained Tween 80 from Sigma-Aldrich in the US. From ACI Pharmaceuticals Ltd. (Bangladesh), we purchased normal saline, Diclofenac sodium, Loperamide, and Diazepam.

Plant identification, collection, and extraction

Approximately 8kg of fresh *Monochoria hastata* leaves were collected from the Hazarikhil Wildlife Sanctuary in Fatikchari, Chittagong Division, Bangladesh (coordinates: 22°40′00″N, 91°40′00″E). The plant material was taxonomically identified and authenticated by Prof. Dr. Shaikh Bokhtear Uddin from the Department of Botany at the University of Chittagong. After identification, the freshly harvested leaves were thoroughly washed and air-dried in the shade for 30 days. Once dried, the leaves were ground using a commercial blender and sieved through a No. 50 test sieve (Endecotts, UK) to obtain a uniform fine powder. For extraction, the powdered material (800 g) was soaked in methanol (3.5 L) and allowed to macerate for 10 days with occasional shaking. Whatman filter paper and a sterile cotton plug were used to filter the mixture, as described by[12]. The filtrate was concentrated using a water bath maintained at 45 °C to obtain the crude methanolic extract. A yield of 1.75% (w/w) was obtained by measuring the percentage from the preliminary weight of the powder.

Experimental animals

Swiss albino mice (aged 6–7 weeks and weighing 20–25 g) were sourced from the Animal Research Facility at Rajshahi, Bangladesh. The mice were acclimatised for 14 days under standard laboratory conditions, with access to food pellets and fresh water, as outlined. Behavioral experiments were conducted between 10:00 a.m. and 3:30 p.m., with a one-hour observation period following each session to assess post-treatment effects. The experimental protocols were approved by the University of Chittagong Animal Ethics Committee [Approval Number: AERB-FBSCU-20250119-(2)] on 10-02-2025 and were conducted in strict accordance with their guidelines.

Treatment design

The mice were randomly assigned to six experimental groups, each consisting of five animals (n=5). The groups included four treatment groups receiving either the methanolic extract (ME) or dichloromethane fraction (DCMF) of *Monochoria hastata* at doses of 200 and 400 mg/kg body weight (administered orally), along with a control group and a standard drug-treated group. Standard treatments consisted of loperamide (5.0 mg/kg b.w., orally) for the castor oil-induced diarrhoea model, diclofenac sodium (10.0 mg/kg b.w., orally) for the analgesic test, and diazepam (1.0 mg/kg b.w., orally) for the anxiolytic test. The oral administration of 10.0 ml/kg b.w. of a 1% Tween-80 aqueous solution was administered to the control group.

Phytochemical investigation

The standard approach[13] was used to identify secondary metabolites in *M. hastata*, including flavonoids, alkaloids, quinones, glycosides, phenols, and other phytochemicals.

Antioxidant activity

The antioxidant activity of the methanolic and dichloromethane fractions of *Monochoria hastata* leaf extract was evaluated by assessing their free radical scavenging capacities against stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) radicals, following established protocols[14].

DPPH radical scavenging assay

The test samples were combined with a 3.0 ml quantity of a solution of DPPH at a concentration that ranged from 15.63 to 500.0 µg/ml. The contents were mixed thoroughly and left to incubate at room temperature for half an hour. After incubation, absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Ascorbic acid, prepared under the same conditions, served as the positive control, while a DPPH solution in methanol was used as the blank. In order to determine the percentage of radical scavenging activity against DPPH, the following formula was used, as demonstrated in[15]:

% DPPH radical scavenging activity=(A−B)/A×100.

Where *A* is the absorbance of the control and *B* is the absorbance of the sample.

Hydrogen peroxide scavenging (HPS) assay

The HPS activity was assessed following the method described by[16]. A 50 mM H_2O_2 solution was prepared in phosphate-buffered saline (PBS, pH 7.4), and 0.6 ml of this solution was mixed with 0.1 ml of the test extract fractions or standard solutions, also prepared in PBS. The mixtures were incubated at room temperature for 10 minutes. Instead of the extract, PBS was utilized as a control. A UV-Vis spectrophotometer was used to measure the absorbance at 230 nm. The material that was used as a reference was ascorbic acid. In order to determine the percentage of radical scavenging activity against hydrogen peroxide, the following formula was used, as demonstrated in:

% hydrogen peroxide scavenging activity=(A−B)/A×100.

Where A is the absorbance of the control and B is the absorbance of the sample.

Antidiarrheal activity by castor oil induced stool count assay

The potential antidiarrheal effects of the Monochoria hastata leaves were evaluated using the castor oil-induced diarrhoea model in mice, following the methodology described by the researcher[17,18], with slight modifications. The Experimental Procedure Section details the arbitrary distribution of five mice to each of six groups; 30 min following treatment, each group of mice received 1.0 ml of castor oil orally to produce diarrhea. Each animal was individually marked on the tail with a permanent marker and placed in a pre-cleaned container lined with a fresh white paper sheet to enable accurate stool counting. The number and consistency of fecal pellets, categorized as wet or dry, were recorded hourly over a four-hour observation period. To maintain consistency and hygiene, the paper lining was replaced at the beginning of each hour. The subsequent formula was employed to determine the percentage inhibition of defecation: Inhibition (%)=[(Fc−Ft)/Fc]×100.

where Fc=mean number of feces of the control group; Ft=mean number of feces of the treated group.

Analgesic activity by acetic acid-induced writhing assay

The analgesic activity of Monochoria hastata extracts was evaluated using the acetic acid-induced writhing test in mice, following the protocol described in a study[19]. The grouping and treatment regimens, including control, standard, and extract-treated groups, were established as outlined in the Experimental Procedure Section. After 45 minutes of treatment administration, each mouse received an intraperitoneal injection of 1% acetic acid to induce nociceptive responses. Five minutes post-injection, the number of writhes (abdominal constrictions) was recorded for each mouse over a 15-minute observation period. The percentage of analgesic activity was calculated using the following formula:

$$\% \text{ Analgesic effect} = [(W_c - W_t)/W_c] \times 100.$$

W_c=No. of writhing in control animals; W_t=No. of writhing in tested animals

Anxiolytic activity

M. hastata's anxiolytic activity was evaluated using the Hole Board Test (HBT) and Elevated Plus Maze Test (EPM).

Hole board test

To further assess the anxiolytic effects of the extracts, the HBT was conducted following established protocols[22,23]. The apparatus consisted of a white wooden platform (20.0×40.0 cm) elevated 15.0 cm above the floor, with 16 uniformly spaced holes across the surface. Grouping and treatment of animals were carried out as per the design described in the Treatment Design Section.

Immediately following the oral delivery of the individual treatments for a period of thirty minutes, each mouse was carefully positioned in the middle of the board and allowed to explore for a total of six minutes. The initial one-minute period was allocated for habituation. During the following five minutes, the number of head-dipping behaviours, defined as the insertion of the head with both eyes passing below the plane of the hole, was manually recorded. An increased frequency of head dipping was interpreted as a reduction in anxiety-related behaviour.

Elevated plus maze test

The EPM test, a validated instrument in behavioral neuroscience, was employed to evaluate anxiety-related behavior[12,20]. The Experimental Procedure Section detailed the arbitrary distribution of five mice to each of six groups. The apparatus was composed of two open arms measuring 50.0 cm by 10.0 cm and two closed arms measuring 50.0 cm by 10.0 cm by 38.0 cm. These arms were placed in a plus shape and were elevated 50.0 cm above the floor. In the middle of the apparatus was a square platform measuring 10.0 cm by 10.0 cm. After gently placing each animal in the middle of the maze with its back to an open arm, they were observed for a period of six minutes. The first minute allowed for acclimatisation, and data were collected during the subsequent five minutes. Observations included the frequency of entries into the open arms and the total time spent therein. These parameters served as behavioral indicators of anxiolytic activity, where increased open-arm exploration suggests reduced anxiety levels[21].

Statistical analysis

The mean±standard error of the mean (SEM) was used to analyse the data. Statistical analysis was conducted using Microsoft Excel (2021) and GraphPad Prism (Version 10.2.3). Statistical decision-making was accomplished through the utilization of a one-way analysis of variance (ANOVA) followed by Dunnett's test and a two-way ANOVA followed by Tukey's test. Statistically significant differences between these values and the control group were declared when the p-value<0.05.

Results

Phytochemicals in *M. hastata*

Preliminary phytochemical analysis of *M. hastata's* methanolic leaf extract revealed various bioactive chemicals (Table 1). The extract was found to contain alkaloids (Wagner test), carbohydrates (Molisch's and Fehling's tests), glycosides (Legal's and modified Borntrager's tests), saponins (foam and Libermann-Burchard tests), phenols, tannins (both via ferric chloride test), flavonoids (alkaline reagent test), terpenes (copper acetate test), phytosterols, and fixed oils (Libermann-Burchard and saponification tests). Proteins and amino acids were the only components that were not detected (xanthoproteic assay).

Table 1 Results of phytochemical screening of methanolic extracts of *Monochoria hastata* leaves

Phytochemicals	Test	Methanolic extract
Alkaloids	Wagner test	+
Carbohydrates	Molisch's test	+
	Fehling's test	+
Glycosides	Legals test	+
	Modified Borntrager's test	+
Saponins	Foam test	+
	Libermann-Burchard test	+
Phenols	Ferric Chloride test	+
Tannins	Ferric Chloride test	+
Flavonoids	Alkaline reagent test	+
Proteins and amino acids	Xanthoproteic test	–
Terpenes	Copper Acetate test	+
Phytosterol	Libermann-Burchard test	+
Fat and fixed oil	Saponification	+

(+)=Present of Phytochemicals and (-)=Absent of Phytochemicals

Antioxidant activity

The MEF and DCMF fractions of *M. hastata* leaves exhibited concentration-dependent DPPH free radical

scavenging activity, as presented in Supplement Figure 1 and Table 2. Among the tested samples, the MEF fraction demonstrated the highest antioxidant potential, with an IC_{50} value of 0.017 µg/mL. This was markedly lower than that observed for the DCMF fraction (0.208 µg/mL) and the standard antioxidant ascorbic acid (1.08 µg/mL). The high R^2 values obtained, 0.8355 for MEF, 0.9678 for DCMF, and 0.9773 for ascorbic acid, indicate a strong linear correlation between concentration and radical scavenging activity. Also, the MEF and DCMF fractions of *Monochoria hastata* leaves demonstrated concentration-dependent HPS activity, as shown in Supplement Figure 2 and Table 2. At a concentration of 500 µg/mL, MEF and DCMF exhibited scavenging activities of 63.56% and 48.31%, respectively, compared to 90.68% for the reference antioxidant ascorbic acid. Interestingly, the DCMF fraction showed a lower IC_{50} value (111.59 µg/mL) than MEF (491.38 µg/mL), indicating greater scavenging potency. However, both fractions were substantially less effective than ascorbic acid, which demonstrated an IC_{50} of 1.08 µg/mL. The high R^2 values observed for MEF (0.9673), DCMF (0.9241), and ascorbic acid (0.9355) reflect a strong and consistent dose-response relationship.

Antidiarrheal activity

Both the ME and DCMF fractions of *M. hastata* leaves exhibited dose and time-dependent antidiarrheal effects in the aforementioned model in mice, as shown in Figure 1. At a dose of 400.0 mg/kg, DCMF achieved the highest inhibition of diarrhoea at the 1-hour mark (76.47%), followed closely by ME (70.59%). The standard drug, loperamide (LPM), produced the most potent effect, with 88.24% inhibition at the same point. Over the 4-hour observation period, the ME-400.0 group demonstrated a sustained inhibitory effect, maintaining 64.71% inhibition at the 4-hour mark. In contrast, DCMF-400.0 showed a gradual decline in efficacy over time. At the lower dose (200.0 mg/kg), both ME and DCMF fractions produced moderate antidiarrheal activity. These findings suggest that while both fractions possess significant dose-dependent antidiarrheal properties, the ME offers more prolonged effectiveness.

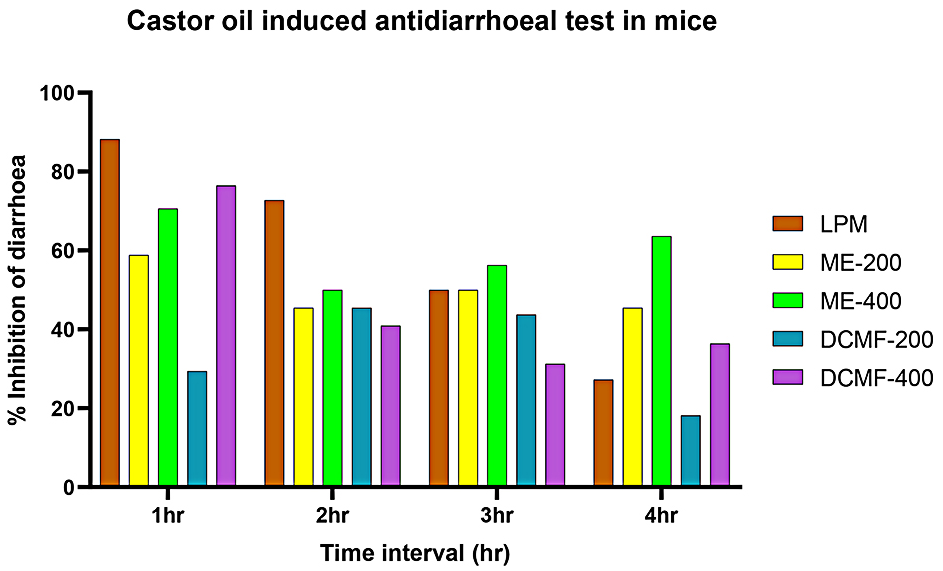
Analgesic activity

The ME and DCMF extracts of *M. hastata* leaves were assessed for peripheral analgesic activity using the aforementioned assay in mice. Both extracts exhibited a dose-dependent reduction of writing, indicating significant analgesic potential (Figure 2A and Figure 2B). Statistical analysis confirmed a strong treatment effect ($F(5, 24)=36.76$, p-value<0.001). ME demonstrated superior activity, with 400.0 mg/kg achieving 56.25% inhibition, comparable to the diclofenac sodium (62.5% at 10.0 mg/kg) and significantly greater than ME-200 (43.98%) and both

Table 2 IC_{50} values and corresponding R^2 values obtained from the DPPH radical scavenging assay and hydrogen peroxide scavenging activity

Sample	DPPH radical scavenging assay		Hydrogen peroxide scavenging activity	
	IC_{50}	R square	IC_{50} (µg/mL)	R square
MEF	0.017	0.8355	491.38	0.9673
DCMF	0.208	0.9678	111.59	0.9241
Ascorbic acid	1.08	0.9773	1.08	0.9355

DPPH=2,2-diphenyl-1-picrylhydrazyl, MEF=methanol extract fraction, DCMF=dichloromethane fraction



LPM=loperamide, ME=methanol extract, DCMF=dichloromethane fraction

Figure 1 Dose- and time-dependent antidiarrheal effects of ME and DCMF fractions of *M. hastata* in castor oil-induced diarrhoea in mice.

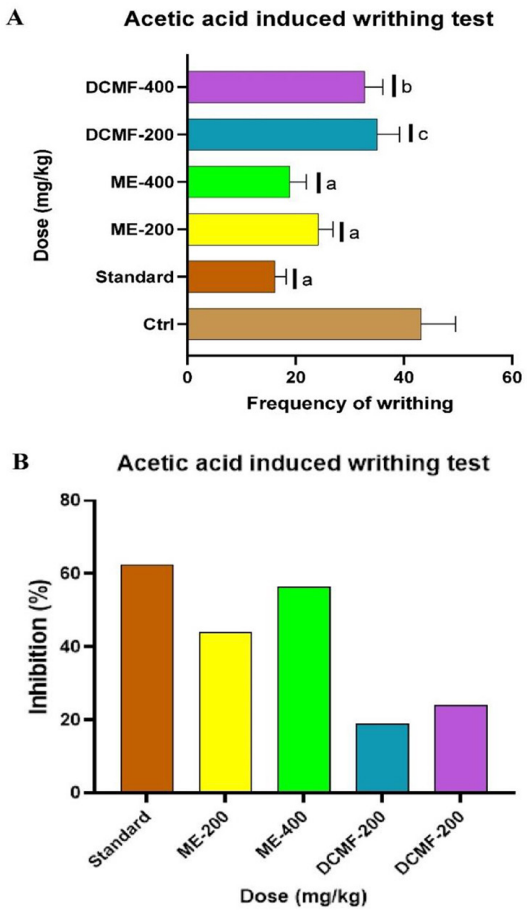
DCMF doses (DCMF-200: 18.75%; DCMF-400: 24.07%). These results suggest that ME, particularly at higher doses, possesses potent peripheral analgesic compounds, while DCMF shows only moderate efficacy. The observed dose-response pattern supports the presence of concentration-dependent bioactive constituents.

Anxiolytic activity

The anxiolytic activity of *M. hastata* leaf extracts was assessed using the HBT, with significant differences observed among groups ($F(5,24)=13.25$, p-value<0.001). Both ME and DCMF fractions increased head-dipping behaviour in a dose-dependent manner (Figure 3). The highest number of head dips was recorded for DCMF-400 (42.8±2.20) and ME-400 (36.6±3.16), with DCMF-400 showing comparable efficacy to the standard drug (45.8±1.85). Lower doses (ME-200 and DCMF-200) exhibited moderate responses (28.8±1.24 and 33.6±2.20, respectively), all significantly higher than the control group (23.4±2.12). In addition, the anxiolytic potential of *M. hastata* leaf extracts was assessed using the EPM test in mice. Significant treatment effects were observed in both the number of entries into open arms ($F(5, 24)=12.57$, p-value<0.001) and the time spent in open arms ($F(5, 24)=303.1$, p-value<0.001) (Figure 4). The reference drug diazepam (1 mg/kg) significantly increased two parameters compared to the control group. Among the extract-treated groups, the dichloromethane fraction at DCMF-400 significantly increased the duration spent in open arms (p-value<0.001), without altering the number of entries. A lower dose of DCMF-200 also increased time spent (p-value<0.05), suggesting a dose-dependent effect.

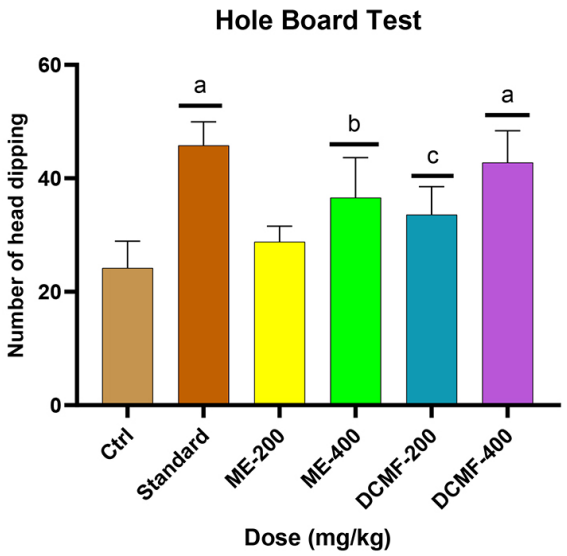
Discussion

Plant-based therapeutics continue to gain interest due to their rich phytochemical composition and multi-target



Ctrl=1% tween-80 vehicle, ME=methanol extract, DCMF=dichloromethane fraction

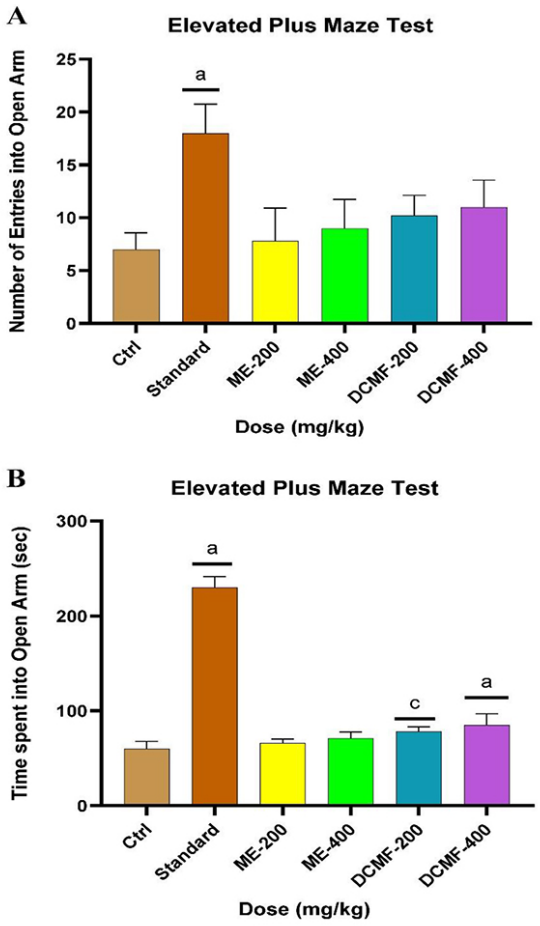
Figure 2 Antinociceptive effects of *Monochoria hastata* leaf extracts in the acetic acid-induced writhing test in mice. (A) Frequency of writhing after treatment with standard (Diclofenac), ME, and DCMF extracts at different doses; values are mean±SEM (n=5). (B) Corresponding percentage inhibition of writhing. Statistical analysis was performed using one-way ANOVA followed by Dunnett's test; different letters indicate significant differences (p-value<0.05) compared to the control.



Ctrl=1% tween-80 vehicle, ME=methanol extract, DCMF=dichloromethane fraction

Figure 3 Effect of *Monochoria hastata* leaf extracts on head-dipping behavior in the Hole Board Test. Mice were treated with Methanol Extract and Dichloromethane fraction extracts at different doses. Values are mean±SEM (n=5); one-way ANOVA followed by Dunnett's test. Different letters indicate significant differences vs. control (p-value<0.05).

pharmacological potential[24]. Among these, antioxidants are an essential component in the process of reducing the effects of oxidative stress, a key factor in various pathophysiological conditions, including inflammation, pain, anxiety, and gastrointestinal disorders[25]. Consequently, antioxidant studies are crucial in understanding how natural compounds can mitigate oxidative stress. In this context, the present study systematically evaluated the antioxidant capacity of the extracts through DPPH and hydrogen peroxide scavenging assays. By calculating the percentage



Ctrl=1% tween-80 vehicle, ME=methanol extract, DCMF=dichloromethane fraction

Figure 4 Anxiolytic effects of *Monochoria hastata* extracts in the elevated plus maze test in mice. (A) Number of entries into open arms. (B) Time spent in open arms (seconds). Mice were treated with Methanol Extract and Dichloromethane fraction extracts at different doses. Diazepam (1 mg/kg) served as the standard anxiolytic. Values are expressed as mean±SEM (n=5); statistical significance determined by one-way ANOVA followed by Dunnett's test. Different letters indicate significant differences vs. control (p-value<0.05).

from the initial weight of the powder, a yield of 1.75 percent (w/w) was obtained. The DPPH assay demonstrated notable radical scavenging activity, indicating that the extracts are rich in hydrogen-donating antioxidants capable of terminating chain reactions initiated by free radicals 26. Notably, ME exhibited significant DPPH radical scavenging activity (IC_{50}=0.017 µg/mL), far exceeding that of DCMF and ascorbic acid, indicating a strong presence of hydrogen-donating phytochemicals such as flavonoids and phenols[27]. The extracts also showed a concentration-dependent hydrogen peroxide scavenging effect, underscoring its potential to protect biomolecules from oxidative damage mediated by non-radical ROS[26]. Interestingly, DCMF showed superior scavenging of hydrogen peroxide radicals, suggesting that different solvent systems isolate distinct antioxidant constituents[28].

This antioxidant activity is particularly important in our context, as it may justify the pharmacological effects observed in our current study, namely the antidiarrheal, antinociceptive, and anxiolytic models, where oxidative stress is known to play a contributory role[29]. The antioxidant activity observed in this study is likely attributable to the presence of flavonoids and phenolic acids in *M. hastata*[30], which are well-documented for their ability to scavenge free radicals and reduce oxidative damage[31]. Previous studies have also reported similar findings, further supporting this conclusion[32].

Extending beyond antioxidant activity, the extracts also demonstrated robust antidiarrheal efficacy in the castor oil-induced diarrhea model. Of note, ricinoleic acid, produced from castor oil through lipase activity, irritates the intestinal lining, leading to the release of mediators such as prostaglandins and nitric oxide that enhance gut movement and fluid secretion[32]. Both ME and DCMF significantly reduced total faecal output in a dose-dependent manner, with the 400 mg/kg dose of DCMF showing inhibitory effects comparable to standard loperamide. This

effect may result from the inhibition of intestinal secretory and motility responses induced by castor oil, potentially mediated by bioactive compounds such as flavonoids and tannins, which are known to reduce fluid loss in diarrheal conditions[33]. These findings align with earlier reports and highlight the potential of M. hastata as a promising natural antidiarrheal agent[34].

In addition to its gastrointestinal effects, M. hastata also exhibited notable analgesic properties, as demonstrated by the acetic acid–induced writhing test. This well–established model evaluates peripheral analgesic activity by simulating localized inflammation through arachidonic acid release and subsequent prostaglandin production, which heightens pain signaling by increasing capillary permeability[35,36]. Thus, compounds that reduce writhing in this model are thought to act primarily by inhibiting the synthesis of prostaglandins, which are among the major inflammatory mediators responsible for eliciting peripheral nociceptive responses[37]. Our findings show that the methanolic extract of M. hastata at 400 mg/kg significantly reduced the number of writhes, indicating strong antinociceptive activity. This effect may be attributed to its high levels of phenolic and flavonoid compounds, which are known for their analgesic and anti–inflammatory properties, further supporting its promise as a natural analgesic agent. These findings are consistent with previous studies, where the crude extract of M. hastata demonstrated approximately 54% pain inhibition in the same model[32]. In silico analyses from earlier reports further support this observation, revealing that the key bioactive compounds in M. hastata, such as rutin and protocatechuic acid, which belong to the flavonoid and phenolic groups, can interact with inflammation–related proteins such as COX–2, LOX, NF–κB, IKBα, and EGFR, helping to reduce pain and swelling[32].

Beyond its peripheral analgesic effects, *M. hastata* leaves also demonstrated promising anxiolytic activity in behavioural models. Both the Hole Board and Elevated Plus Maze Tests revealed that DCMF significantly increased exploratory behavior and time spent in open arms, indicating reduced anxiety. To our knowledge, no prior research has examined the anxiolytic effects of *M. hastata*. However, potential insights can be drawn from related plant species and known mechanisms of herbal anxiolytics. As many of these plants reduce anxiety by modulating the GABAergic system, maintaining the balance between excitatory and inhibitory signals in the brain, and reducing inflammation, mechanisms are also targeted by standard anxiolytic drugs like diazepam[12]. These findings, along with the traditional use of *M. hastata*, suggest its potential in anxiety management, though direct evidence and further research are necessary to validate its efficacy and underlying mechanisms.

Taken together, the findings of this study highlight the multifaceted pharmacological potential of *Monochoria hastata*, demonstrated through its antioxidant, antidiarrheal, analgesic, and anxiolytic activities. These effects are likely mediated by its rich phytochemical profile, particularly flavonoids and phenolic acids, which interact with key biological targets involved in oxidative stress, inflammation, and neurotransmission. Although direct evidence for certain activities, such as its anxiolytic effects, is still emerging, the observed outcomes, supported by the literature on related species and mechanistic insights, underscore *M. hastata* as a promising candidate for further investigation and development as a plant–based therapeutic agent.

Conclusion

This study provides strong evidence that *Monochoria hastata* leaves have ethnomedicinal relevance by showing that its ME has excellent antioxidant and analgesic properties (56.25 percent inhibition of writhing at 400 mg/kg) and that the DCMF has significant antidiarrheal and anxiolytic effects (like diazepam in the hole–board test). The bioactivity of the plant's ingredients is revealed by

its solvent-dependency; this property emphasizes the antioxidant and analgesic activities of ME and the efficacy of DCMF against diarrhea and anxiety. Further isolation of bioactive chemicals and mechanistic studies should be conducted to enhance drug development. These results establish *M. hastata* as a prospective multi-target therapeutic candidate for oxidative stress, gastrointestinal problems, pain, and anxiety.

Author contributions

Conceptualization, S.M. Naim Uddin; methodology, Sanjida Ilias Shemoon, and Md. Hossain Rasel; software: Rakibur Rahman, and Sanjida Ilias Shemoon; investigation: Sanjida Ilias Shemoon, and Md. Jahirul Islam Mamun; resources: S.M. Naim Uddin, and Mir Mohammad Nasir Uddin; formal analysis: Rakibur Rahman; visualization: Rakibur Rahman; data curation: Mir Mohammad Nasir Uddin, and S.M. Naim Uddin; writing—original draft preparation: Rakibur Rahman, and Sanjida Ilias Shemoon; writing—review and editing: Mir Mohammad Nasir Uddin; supervision: S.M. Naim Uddin.

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Conflict of interest

A declaration was made by the authors stating that there was no conflict of interest.

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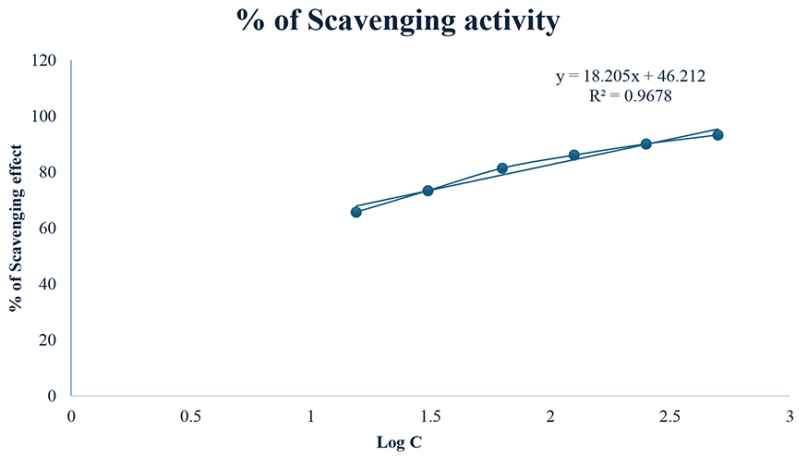
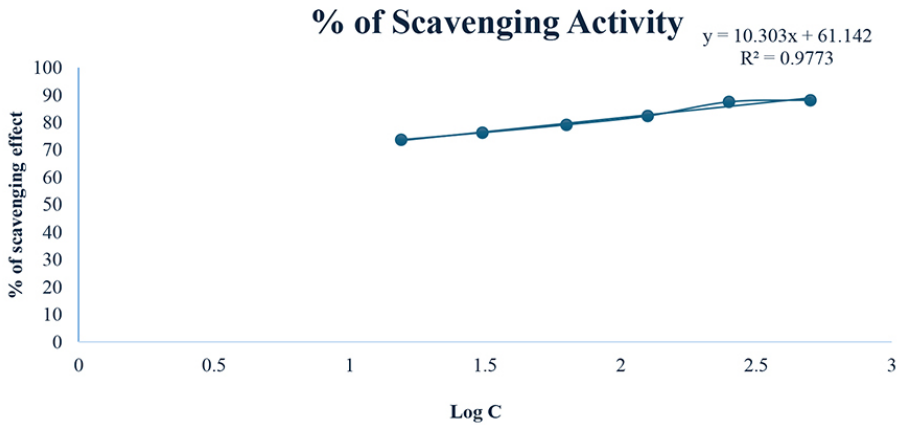


Supplementary Figure 1 antioxidant activity of Ascorbic Acid (AA) by calculating % of scavenging effect and IC_{5O} Value using DPPH free radical scavenging assay.



Supplementary Figure 2 Antioxidant activity of different concentration of crude Methanol extract of *Monochoria hastata* leaves by calculating % of scavenging effect and IC_{50} value using DPPH free radical scavenging assay