Evaluation of in-vitro anthelmintic and in-vivo analgesic and antidepressant activities of methanolic extract of *Pseuderanthemum latifolium*

ABSTRACT

Background: *Pseuderanthemum latifolium* is a species of the Acanthaceae family. In Bangladesh, Nepal, and India, the plant is traditionally used to treat asthma, reduce pain, infection, and other lung problems.

Objectives: This study aims to pave the way for future studies on the analgesic, antidepressant, and anthelmintic properties of *P.latifolium* methanolic extract.

Materials and Methods: The analgesic activity is evaluated employing the acidic acid-induced writhing test & formalin-induced paw licking test on Swiss albino mice. The plant extracts antidepressant efficacy is tested by using forced swimming and tail suspension techniques following in-vitro anthelmintic efficacy along with its qualitative phytochemical analysis.

Result: Conventional phytochemicals was found out to be present during the phytochemical test. Anthelmintic test determined that the anthelmintic activity was directly proportional to the concentration of the extract. In the acetic acid-induced writhing test, the writhing response significantly (40.28% and 46.75%) inhibited compared to diclofenac sodium. At 400 mg/kg, the extract substantially reduced the formalin-induced paw licking response in both the early (32.24%) and late phases (22.56%). The antidepressant effect of MEPL in Swiss albino mice was assessed by the immobility times, where it was 108.67 (200 mg/kg) and 110 (400 mg/kg) sec in the forced swimming test and 92.67 (200 mg/kg) and 79.33 (400 mg/kg) sec in the tail suspension method. The standard in both models was 20 mg/kg of fluoxetine.

Conclusion: The current study's findings laid the groundwork for additional research into the therapeutic potential of *P.latifolium* methanol extract in analgesic, antidepressant, and anthelmintic activity.

Keywords: P. latifolium, analgesic, antidepressant, anthelmintic activity.

INTRODUCTION

Plants have traditionally played an important part in illness therapy. Every plant has unique phytochemicals that have a wide range of therapeutic properties. Plants are rich in medicinal ingredients; great emphasis has been paid to the creation of ethnomedicines because they include phenols, flavonoids, flavonols, alkaloids, tannins, proanthocyanidins, vitamins, terpenoids, and other phytochemicals responsible for various pharmacological effects [1]. These compounds show a variety of functions that can be used to create medications that are both more effective and less likely to cause adverse side effects than the existing state of abnormal health in humans or other animals [2]. Growing concerns about helminths developing anthelmintic resistance led to the notion of evaluating medicinal plants for anthelmintic action. WHO has established a target of regularly treating at least 75% of school-age children in endemic areas with anthelminthic medication [3]. In addition to interfering with our day-to-day activities, pain increases rates of job absence, underemployment, and unemployment, which costs the person who is suffering it a significant amount of money [4]. It is commonly accepted that opioids alleviate pain by acting on the central nervous system via three opioid receptors (ε, κ, and δ). When treating chronic pain, these drugs are especially helpful. Non-steroidal anti-inflammatory medicines (NSAIDs) are recommended for the treatment of mild to moderate pain, whereas steroidal and opioid medications are used to treat acute and chronic pain [5]. Depression is a common disorder, which is a recurrent mental disease characterized by a negative mood and loss of interest or pleasure in the normal activities of daily normal life. It is often attended by a broad range of symptoms, including a decline in cognitive function, sleep disturbance and recurrent suicidality, which affect the quality of life and mortality of the affected patients [6]. Numerous medications are effective in treating anxiety and depression. While benzodiazepines are helpful for treating anxiety and muscular relaxation, tricyclic antidepressants, monoamine oxidase inhibitors, serotonin-norepinephrine reuptake inhibitors, and serotonergic antidepressants are the main medications used to treat depression [7-8]. These drugs have a number of positive benefits, but they can also have negative consequences. Because of this, some scientists are concentrating on researching medicinal plants in an effort to identify compounds that are just as powerful but less harmful. The World Health Organization (WHO) reports that around 75% of the world's population receives their medical care or treatments from traditional medicine practitioners [9].

In this study, we evaluated the medicinal activities of *Pseuderanthemum latifolium,* which is a species of the Acanthaceae family [10]. P. latifolium leaves were used to cure a variety of illnesses, including trauma, hemorrhoids, inflammation, wound healing, and bleeding [11].

Fresh P. latifolium leaf water extract also had bradycardic and hypotensive properties [12]. Therefore, the purpose of our current study was to examine the depressive, analgesic, and in-vitro anthelmintic properties of *Pseuderanthemum latifolium* methanolic extract.

MATERIALS AND METHODS

Collection and identification of plant material

In April 2022, the plant *Pseuderanthemum latifolium* was obtained from the Hazarikhil hill tract region of Fatickchari Chattogram District, Bangladesh. Dr. Shaikh Bakhtiar Uddin, Professor in the Department of Botany at the University of Chittagong in Bangladesh, identified the plant.

Preparation of extract

The plant was dehydrated for 14 days in the dark on the ground. The crushed plant (200 mg) was saturated with an appropriate amount of methanol and kept at room temperature for twelve days with inconsistent shaking and stirring. The entire mixture was then filtered, and the resultant filtrate was concentrated into a sticky material, using a rotating evaporator. The sticky stuff was placed in storage. Temperature under a ceiling fan for extract drying (about 7%).The whole plant extract was prepared for antidepressant and anxiolytic action testing.

Experimental animals

Swiss albino mice weighing between 18 and 30 grams were obtained from the animal facility at Chittagong Medical College in Chattogram, Bangladesh. The mice were given lab-standard food and filtered water ad libitum and kept in a room with adequate ventilation and a natural day-night cycle. All studies were done in a calm, secluded setting. The study protocol was cleared by the P&D Committee of the Department of Pharmacy at International Islamic University Chittagong in Bangladesh. The mice were habituated to laboratory conditions for seven days before the study.

Chemicals and equipment

The following drugs and chemicals were used in this study**:** Diazepam (Square Pharmaceuticals Ltd, Bangladesh), fluoxetine (Albion Laboratories Ltd, Bangladesh). Diclofenac sodium (Beximco Pharmaceuticals Ltd., Dhaka, Bangladesh), formaldehyde (MERCK, Mumbai, India), acetic acid (MERCK, Mumbai, India), normal saline solution (0.9%

NaCl) and Tween-80 (BDH Chemicals, UK) were used. All other reagents were of analytical grade.

Phytochemical screening

Standard phytochemical techniques were used to qualitatively analyze the freshly synthesized MEPL for alkaloids, carbohydrate, protein, tannins, reducing sugar, flavonoids, and phenol.

Anthelmintic activities

The anthelmintic activity of MEPL was measured using a method that had been used before. In this study, an aquarium worm (*Tubifex tubifex*) was used for the test because it is similar to an intestinal worm (Annelida) in terms of how it works and how it looks. The worms were bought at an aquarium shop in Chittagong, Bangladesh. The average length of the worms used in the experiment was between 2 and 2.5 cm. In this case, the test was done three times and randomly split into five groups: Group I, which only used distilled water, was a bad control.

As a positive control, Group II used the standard drug Albendazole (1 mg/mL). Groups III, IV, and V were used as test groups, each with a different amount of MEPL (5, 8, or 10 mg/mL). In this study, 10 to 12 worms were put in each Petri dish, and 3 mL of MEPL extract solution with different concentrations was added to each group. Then, the times the worms started, when they stopped moving, and when they died were carefully watched and written down. The anthelminthic activity was measured at two different times: when the worms were paralyzed and when they died. The paralyzing time was measured by how long it took for the worms to stop moving after being shaken hard. After making sure that the worms didn't move when shaken hard or put in slightly warm water, the time of death was written down. Linear regression was used to figure out what the best concentration of MEPL was for killing worms on *Pseuderanthemum latifolium* compared to standard Albendazole (1 mg/mL) [13].

In vivo analgesic activity

Acetic acid-induced writhing test

A total of four groups, each including three mice, were formed out of the mice. In order to conduct the writhing test, a 0.6% (v/v) acetic acid solution (10 mL/kg body weight) was given intraperitoneally to each mouse. After 20 minutes, the number of writhing and stretching movements was recorded [14] [15]. The first group was used as a control and was given 1% Tween in an amount equal to 10 milliliters per kilogram of body weight. The second group

was given diclofenac sodium in an amount equal to 10 milligrams per kilogram of body weight as a standard orally treated with methanol extract at doses of 200 and 400 mg/kg for thirty minutes before receiving an injection of acetic acid. The level of analgesic activity, measured in terms of the percentage of writhing that was inhibited, was determined for each animal by applying the following formula:

Writhing inhibition (%) $W_C - W_S / Wc = \times 100$

Where, Wc is the mean number of writhing's of the control and Ws is the mean number of writhing's of the test sample.

Formalin induced paw licking method

The 20 µL of 2.5% formalin in saline was injected subcutaneously to a hind paw of the mice after 30 minutes of receiving diclofenac sodium 10 mg/kg (group 2), the third and fourth groups received a methanol extract p.o. dose of 200 and 400 mg/kg, respectively. During the experiment, the first group (serving as a control) was given only formalin (20 µL at a 2.5% concentration). Data were expressed as the total amount of time the injected paw was licked in the early phase (between 0 and 5 minutes) and the late phase (between 15 and 30 minutes) after the formalin injection, which was considered an indicator of the pain response. A timer was used to measure how long the dog spent licking or biting at the injured paw (pain reaction). Similar to how the earlier calculation for both acute and chronic pain phases determined the percentage of pain inhibition, this one may be used to estimate the percentage of pain suppression [14] [16].

Preparation of test doses

The extracts were suspended in 1% Tween 80 solution. Various concentrations were prepared from a stock solution (40 mg/mL). The solutions were freshly prepared to administer orally.

In vivo antidepressant activity

Tail suspension test

Steru et al. developed the TST [17], which is now widely utilized as a behavioral paradigm for assessing the antidepressant activity in mice. The mice were brought from their housing colony to the laboratory in their own cages, and once they were there, they were examined. They were given one to two hours to become acclimated to the conditions of the laboratory. Mice in Groups III and IV were given MEPL by oral administration at doses of 200 and 400

mg/kg body weight, respectively. Group I was used as a control and received 1% Tween 80 at a rate of 10 milliliters per kilogram of body weight (p.o.). Group II was given the standard drug, which was 20 milligrams per kilogram of body weight of fluoxetine. By placing adhesive tape around one centimeter from the end of each mouse's tail and attaching it to the rim of a table at a height of fifty centimeters above the floor, each mouse was individually hung in midair. During the course of the experiment, each mouse was kept acoustically and visually separate from the other mice. A stop watch was used to manually record for six minutes the entirety of the time spent standing still. Immobility in mice was defined as the absence of any visible bodily movement as well as a state of complete and utter passivity. Mice were regarded as immobile when they hung completely still. The experiment was carried out in a room with low lighting, and each mouse was only utilized once for the duration of the test. The observer responsible for recording the immobility of mice was unaware of the pharmacological treatments that were administered to the mice that were being tested [15].

Forced swim test (FST)

The FST, which was initially developed by Porsolt et al. [18], is commonly utilized as a behavioral model for the purpose of assessing antidepressant-like activity in rodents. In this technique, mice were kept in an open glass box that measured 25 centimeters by 15 centimeters by 25 centimeters and contained fresh water up to a height of 15 centimeters. The temperature was kept at 261 degrees Celsius. Due to the high level of water, mice were unable to support their bodies by pressing their hind paws or tails on the base or side walls of the compartment. After subjecting each mouse to the forced swimming test, the water in the enclosure was changed because it has been proven that "used water" can influence the behaviors of the animals. During the first two minutes of the test, every mouse showed a high level of activity in terms of its movement. During the remaining four minutes of the entire testing time of six minutes, the duration of the subject's inactivity was manually recorded. It was determined that the mouse had become immobile when it stopped struggling and continued to hang motionless in the water, doing just the activities required to maintain its head above water. After that, the mice were cleaned off and put back in their original cage [19][15].

RESULTS

Phytochemical screening

The preliminary qualitative analysis revealed that the *Pseuderanthemum latifolium* plant is rich in alkaloid, flavonoid, carbohydrate and protein (Table-1).

Table 1: Result of phytochemical group test of the extracts of *Pseuderanthemum latifolium*

Sample	Alkaloids	Carbohydrates	protein	Quinones	Tannins	Flavonoids	phenol
MEPL	+++	+++	+++	-	-	++	-

+ = present; − = absent

Anthelmintic activities:

The anthelmintic activity of the MEPL is illustrated in Table 2. From the lowest to the highest concentration (5, 8, and 10 mg/mL), the extract's anthelmintic activity was found to be directly correlated with the concentration used. MEPL exhibited substantial paralysis periods of 60.33 min, 51.67 min, and 35 min at concentrations of 5, 8, and 10 mg/mL, whereas times to death were 85 min, 67 min, and 57 min, respectively. The positive control (Albendazole, 1 mg/mL) in the experiment had a paralysis time of 51.33 minutes and a time to death of 66.67 minutes.

Table 2: Anthelmintic activity of methanol extract of MEPL

Concentration(mg/ml)	Paralyze Time (min)	Death time(min)
5	60.33	85
8	51.67	67
10	35	57
Control(water)	0	0
Albendazole (standard)	51.33	66.67

Analgesic activity assay

Acetic acid-induced writhing test

By acetic acid-induced abdominal writhing test when statistically analyzing the recorded data, it was seen that MEPL produced an exceptionally significant reduction of writhing ($p < 0.05$) at the 200 and 400 mg/kg doses when compared with the control group. This supports the significant analgesic activity of this extract. (Table-3)

Table 3: Analgesic activity of MEPL of plant *Pseuderanthemum latifolium* on acetic acid-induced mice.

Group	Writhing (mean ± SEM)	% of inhibition
Control	25.2 ± 0.52	0
Standard	6.2 ± 0.33	75.4
MEPL-200	15.2 ± 0.76	39.68
MEPL-400	13.4 ± 0.54	47.62

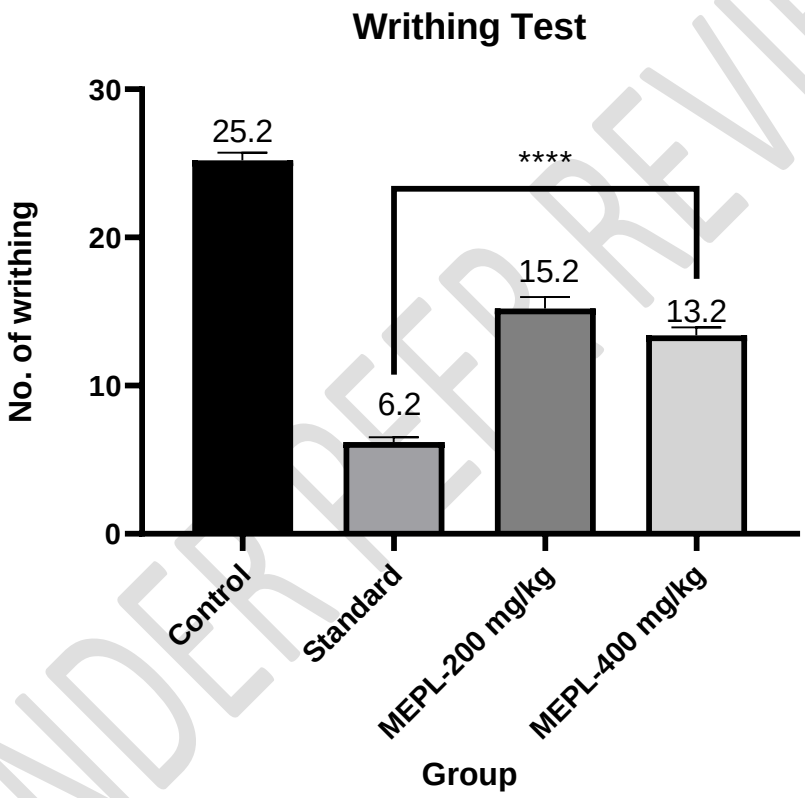


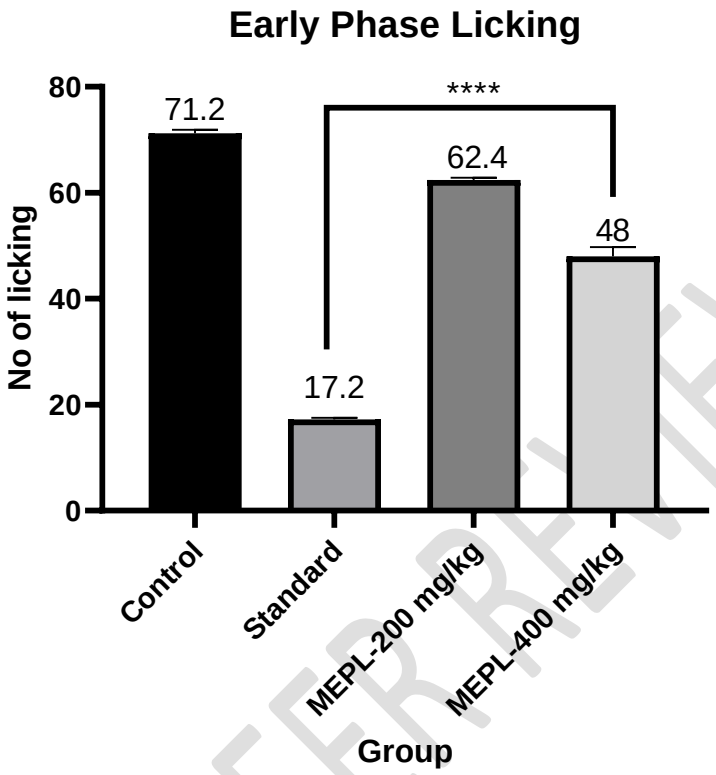
Figure-1: Analgesic activity (No. of writhing) of methanol extract of *Pseuderanthemum latifolium* leaves and diclofenac sodium in acetic acid induced writhing test. The outcomes have been manifested as mean ± SEM (n = 5) with the statistically significant ($P < 0.05$) in comparison to the positive control group processed by using one-way ANOVA analysis (Graph pad Prism software, Version 8.4.1) for multiple comparisons.

Formalin-induced paw licking test

The formalin-induced paw licking test results showed that MEPL, at a dose of 400 mg/kg, significantly inhibited the licking in both phases, with inhibitions of 32.58% at the first phase and 23.08% at the last phase, whereas diclofenac, the standard drug, significantly inhibited the reflex in both phases at a dose of 10 mg/kg body weight, with inhibitions of 75.84% and 62.9%, respectively. The extracts' analgesic efficacy was notable when compared to the control. The complete results are displayed. (Table-3)

Table-4: Effect of the extracts of *Pseuderanthemum latifolium* on formalin-induced paw licking in mice.

Treatment	Early phase licking (mean ± SEM)	% of Inhibition	Late phase licking (mean ± SEM)	% of Inhibition
Control	71.2 ± 0.66	0	44.2 ± 0.33	0
Diclofenac Na (10mg/kg)	17.2 ± 0.33	75.84	16.4 ± 0.46	62.9
MEPL-200	62.4 ± 0.46	12.36	39.2 ± 0.52	11.31
MEPL-400	48 ± 1.72	32.58	34 ± 0.63	23.08



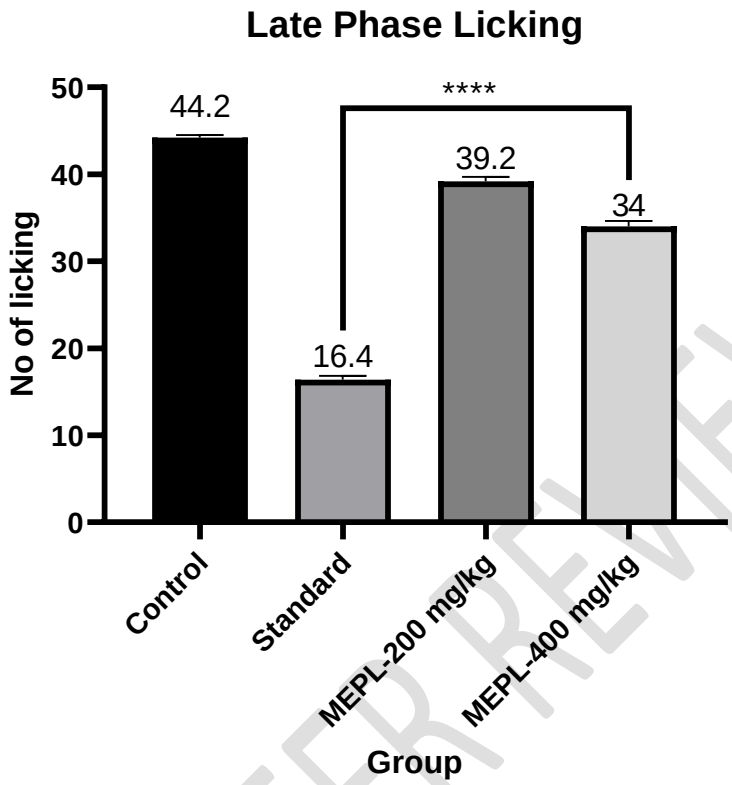


Figure 2: Analgesic activity (paw licking time in sec) of methanol extract of *Pseuderanthemum latifolium* leaves and diclofenac sodium in formalin induced paw licking test. The outcomes have been manifested as mean ± SEM (n = 5) with the statistically significant ($P < 0.05$) in comparison to the positive control group processed by using one-way ANOVA analysis (Graph pad Prism software, Version 8.4.1) for multiple comparisons.

Antidepressant activity

Tail suspension test

In this experiment, mice administered with two doses of *Pseuderanthemum latifolium* extract (200mg/kg and 400 mg/kg) showed significant ($p < 0.05$) reductions in immobility periods, which was significant when compared to the negative control. Among the study treatments, the 400 mg/kg methanolic extract had the strongest antidepressant activity and the shortest

immobility duration (81.4 ± 2.63 s). Similarly, mice injected Fluoxetin (10 mg/kg) demonstrated a significant decrease in immobility time (49.2 ± 0.66 s).

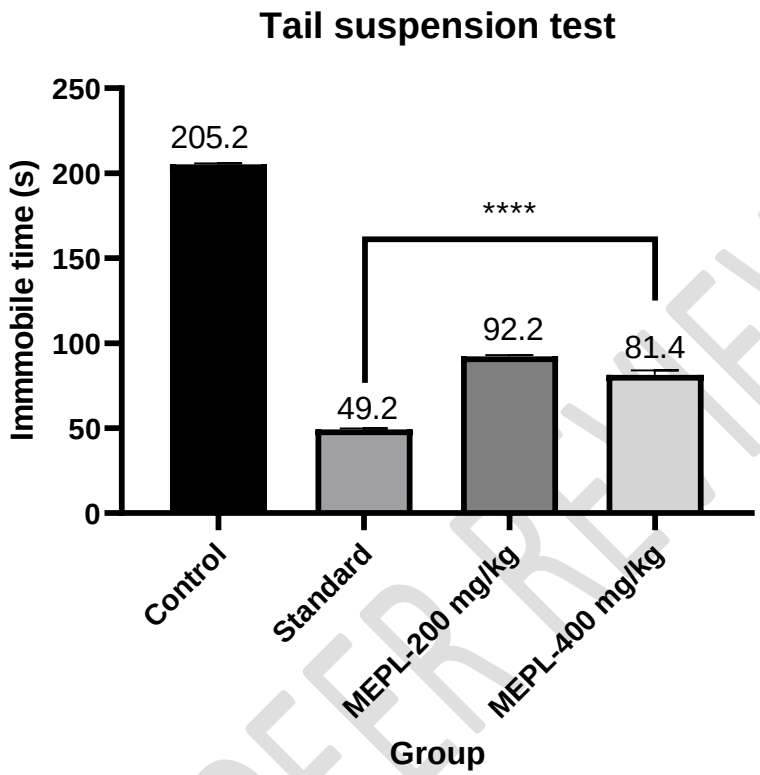


Figure 3: Antidepressant activity (immobility time) of MEPL and diazepam in tail suspension test. The outcomes have been manifested as mean ± SEM (n = 5) with the statistically significant ($P < 0.05$) in comparison to the positive control group processed by using one-way ANOVA analysis (Graph pad Prism software, Version 8.4.1) for multiple comparisons.

Forced swimming test

The forced swimming test was used to investigate the possible antidepressant effects of MEPL following oral treatment. In this test, mice administered with two doses of every sample (200 and 400 mg/kg), immobility times showed reductions in their immobility, which were significant compared with the positive control (153.1 ± 1.43). MEPL at the 200mg/kg dose showed the highest amount of antidepressant activity, where it decreased immobility time (108.6 ± 5.35 s). Similarly, mice treated with Fluoxetine (10 mg/kg), as expected, showed a significant decrease in immobility time (82.8 ± 3.55 s).

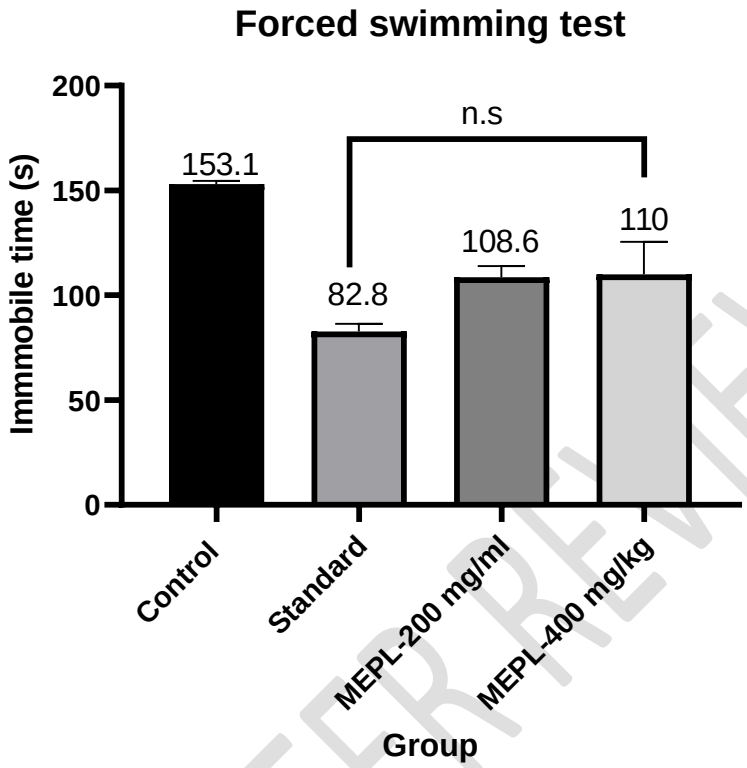


Figure 4: Antidepressant activity (immobility time) of MEPL and diazepam in forced swimming test. The outcomes have been manifested as mean ± SEM (n = 5) with the statistically significant ($P < 0.05$) in comparison to the positive control group processed by using one-way ANOVA analysis (Graph pad Prism software, Version 8.4.1) for multiple comparisons.

DISCUSSION

New medications with therapeutic benefits for humans may be derived from plants [20]. The Food and Drug Administration (FDA) has approved a large number of medications today, many of which have botanical origins. A wide range of substances found in plants used in traditional medicine can be utilized to treat infectious and chronic illnesses. Alkaloids, carbohydrates, proteins, and flavonoids were found in the phytochemical examination of the P. latifolium preparations.

For this study, however, the plant used was P. latifolium, an important ethno medicinal plant with a wide range of medical properties. The current study's results, obtained using both in vivo and in vitro methodologies, demonstrated the powerful and efficient anthelmintic, analgesic, and antidepressant activities of P. latifolium methanol extract. The aquarium worm *Tubifex tubifex* was used in an anthelmintic study which is a suitable host for the *Myxobolus cerebralis* parasite [21]. The results suggested that MEPL showed a dose-dependent manner significant reduction in paralysis and death time of the worm, indicating the possible mechanism of anthelmintic activity. The Alvendazole is used as a standard drug in different concentrations, which is a nicotinic receptor agonist. It acts by activating the nicotinic acetylcholine receptors on the muscle of the worm resulting in paralysis and death [22]. The different concentrations (5, 8, and 10 mg/mL) of MEPL exhibited a directly proportional anthelminthic activity, whereas the 10 mg/mL showed a similar mechanism like Alvendazole 1 mg/mL. The current result was suggesting that MEPL could be used or formulated as an anthelminthic agent.

In addition to testing for anthelmintic activity, the present investigation used animal behavioral models to determine if MEPL had antidepressant effects. The TST and FST are commonly used devices to evaluate antidepressant effects in animals. Both assays are sensitive enough to measure the effects of any type of antidepressant medication (such as tricyclics, monoamine oxidase inhibitors, and selective serotonin reuptake inhibitors) [4-5]. Interestingly, antidepressant drugs have been shown to decrease the immobility duration in rodents—a unique behavior that is assessed in this study and is believed to indicate behavioral discomfort similar to that seen in depression in humans [25]. In both of the classical models employed in this investigation, treatment with MEPL showed an antidepressant-like effect, and the outcomes were found to be almost identical to those of the widely used drug fluoxetine. In the current investigation, administration with MEPL at varied dosages has been observed to reduce the time of immobility in both FST and TST. The 400 mg/kg extract dose in our investigation resulted in larger decreases in immobility, comparable to those seen with the conventional medication fluoxetine.

The researcher claims that behaviors resembling depression are caused by the GABA-A receptor. Depression may occur in patients who are both deficient in GABA and exhibit a lower level of the GABA-A receptor. Therefore, medications that imitate the GABAergic receptor may be useful in lessening the intensity of depression [26]. The observation that P. latifolium extracts exhibit antidepressant properties might perhaps be attributed to GABAergic system activation.

The anti-nociceptive activity of MEPL was assessed using the acetic acid-induced writhing test, which produced turning of the dorso-abdominal muscles, contractions of the abdomen, and overall body movements. By stimulating the nociceptive neurons, acetic acid in this approach helps to trigger the expulsion of inflammatory agents such as bradykinin and serotonin [27]. The writhing brought on by acetic acid was significantly reduced by the intraperitoneal injection of MEPL. The formalin test is susceptible to various moderate analgesics and nonsteroidal anti-inflammatory medications [28]. A chemical nociceptive stimulation that causes a reflexive reaction suggestive of pain is used in the test. The two distinct stages of the test may represent the various forms of pain [9-10]. The early stage may result from direct actions on nociceptors, and centrally active analgesics, like morphine, can prevent it. On the other hand, nonsteroidal anti-inflammatory medications, steroids, and centrally acting medications can all suppress the late phase, which may be related to an inflammatory response that is partially mediated by prostaglandins. It is feasible to clarify the impact of inflammation on the reactions in the two phases since it happens at the location of the formalin injection. In the late phase of our investigation, a small inhibitory impact was seen in comparison to the reference medication, diclofenac sodium, indicating a comparatively milder central anti-nociceptive action of the extract. MEPL treatment at varied dosages significantly reduced pain response across all stages, as seen by decreased licking behavior. This biphasic lowering of liking behavior is a marker for neuronal and inflammatory pain regulators.

CONCLUSION

The current study investigated the anthelmintic, analgesic, and antidepressant activities of a methanolic extract of *Pseuderanthemum latifolium*. MEPL has shown potent analgesic and antidepressant properties. The data strongly imply that the extract may include molecules capable of displaying such capabilities. As a result, more study is needed to identify the specific compounds and gain a better knowledge of the mechanism underlying such an action in the *Pseuderanthemum latifolium* plant.

FUTURE IMPLICATION AND LIMITATIONS OF STUDY

Our findings show that the methanolic leaf extract of *Pseuderanthemum latifolium* has strong anthelmintic activity as well as mild-to-moderate analgesic and antidepressant properties. The study offers possible future applications in pharmaceutical development in mental health therapies and pain management. However, limitations such as animal model specificity, a single dosage and administration route, short-term effects, and a limited scope of neuropharmacological effects are highlighted, emphasizing the need for dose-response

studies, clinical trials, mechanistic studies, long-term investigations, and bioactive compound exploration to enhance the translational potential of research findings and facilitate their practical applications in medicine and healthcare.

DECLARATIONS

Ethics approval and consent to participate: The Institutional Animal Ethical Committee of the School of Pharmacy at the International Islamic University Chittagong in Bangladesh granted approval for the research (reference number [P&D-147/13-18]). For ethical reasons, each animal was used only once and all animals were sacrificed at the end of the study.

Consent for publication: Not applicable.

Availability of data and material: The datasets supporting the conclusions of this article are included within the article.

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