Original Article

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Phytochemical Profiling and Pharmacological Evaluation of the Methanol Extract of *Persicaria chinensis L.H. Gross* Leaves for Thrombolytic, Antidepressant, and Anxiolytic Activities through the *In Vitro* and *In Vivo* Approach

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Abstract:

Objective: *Persicaria chinensis L.H. Gross* is an herbal member of the *Persicaria* genus and the *Polygonaceae* family. The objective of this study was to analyze the thrombolytic, antidepressant, and anxiolytic activities of the methanol extract of *Persicaria chinensis* leaves.

Materials and Methods: The current study uses several different experimental methods to investigate the presence of bioactive metabolites and the roles that *Persicaria chinensis* methanol leaf extract (MEPCL) plays in reducing thrombosis, anxiety, and depression. Blood was withdrawn from volunteers who underwent an *in vitro* examination to assess thrombolytic activity. The anxiolytic effects were observed by employing elevated plus maze (EPM) and hole-board (HBT) tests, while the antidepressant effects were evaluated using forced swimming (FST) and tail suspension tests (TST).

Results: Alkaloids, steroids, saponins, glycosides, phenols, tannins, flavonoids, terpenoids, quinones, and reduced sugar are the secondary metabolites present in MEPCL, which was the preliminary investigation of this study. The MEPCL showed mild thrombolytic activity. The immobility duration of both doses of MEPCL was significantly decreased in TST and FST. In HBT, the higher doses showed a considerable increase in head dipping, and, in the EPM test, time spent in the open arm was increased significantly with an increased dose.

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Conclusion: This herb has potential for managing thrombosis, anxiety, and depression-like disorders.

Keywords: anti-depressant, anxiolytic activity, *Persicaria chinensis*, phytochemical screening, thrombolytic

Introduction

Plants were present in the early geological era of the Earth's history. Plants have been used to relieve human pain for as long as human civilization has existed[1]. Plants with medicinal qualities or "Medicinal Plants" are typically defined as those that have positive pharmacological effects on the bodies of animals and humans. Plants with therapeutic characteristics are those that generally integrate certain secondary metabolites (e.g., tannins, alkaloids, glycosides), and include both vitamins and minerals[2]. According to estimates from the World Health Organization (WHO), almost 80 percent of people worldwide utilize herbal medicines for some form of basic healthcare. Approximately 5,000 different kinds of medicinal plants exist; these are mentioned in the "Materia Medica"[3]. The therapeutic potential and extensive phytochemical profiles of medicinal plants have long made them essential in managing a wide range of pathological disorders. According to recent research, plant-derived substances can effectively reduce intestinal mucositis brought on by chemotherapy and modify cancer-related microRNAs through nutritional immunomodulation[4].

Thrombolysis is a medical procedure intended to preserve tissues and organs, improve blood flow, and break up dangerous clots in blood vessels. When blood clots develop in the arteries feeding the heart and brain or in the arteries supplying the lungs, thrombolysis is commonly employed as an emergency treatment. Thrombosis can impede blood flow to tissue, which can lead to ischemia and tissue death[5]. Thrombolytic drugs can break down blood clots. A method called "thrombolytic therapy" can also be performed to relieve the symptoms caused by a blood clot and restore function to the area that has been invaded. Anticoagulant medications are prescribed to patients who have a high risk of thrombosis; nonetheless, these medications may intentionally or unintentionally alter the body's coagulation factors[6].

Neuropharmacology is the study of how drugs alter the characteristics of nervous system cells. Most neuropharmacological treatments focus on physiological or synaptic methods, which are associated with synaptic activation. According to estimates from the World Health Organization, the number of people afflicted by neurological disorders worldwide is one billion, with 50 million people suffering from epilepsy and 24 million people suffering from dementias such as Alzheimer's. It is estimated that these diseases are responsible for 6.8 million deaths within the United States each year. Depression is a prevalent and possibly hazardous mental illness that alters one's feelings, thoughts, and behavior. Depression is typified by melancholy or a decline in interest in once-valued pursuits. It can result in a variety of physical and psychological issues[7]. People use antidepressants to treat major depressive disorders, anxiety disorders, anorexia nervosa, severe pain, addiction, insomnia, and other disorders. Numerous controlled clinical studies conducted over the past 50 years have shown that antidepressants work better than placebos[8]. This class of antidepressants (originally identified in the 1950s) selectively inhibits the enzyme monoamine oxidase, which causes reduced monoamine breakdown. Anxiety disorders arise when a person's response deviates greatly from what would be anticipated in a particular circumstance. It is among the most common mental health conditions[9].

Persicaria chinensis L.H. Gross is the common name for Chinese knotweed, a herb species from the Polygonaceae family[10]. It is present in a wide range of

countries, including the Indian Subcontinent, Indonesia, Malaysia, and Myanmar. People in Malaysia and Vietnam, where it grows in abundance, use it to prepare herbal treatments[10]. Traditional Chinese medicine has employed *Persicaria chinensis* to alleviate ulcers, eczema, stomach pains, and multiple inflammatory skin diseases[11]. Its medicinal uses include the alleviation of diarrhea, enteritis, and sore throats through herbal treatments[11]. Externally, people employ the decoction to alleviate aural eczema. People have used the herb's juice to treat ocular diseases[11]. Several prior studies assessed the antibacterial and antifungal properties of *Persicaria chinensis L.H. Gross*[12].

The literature review revealed that the leaves of *Persicaria chinensis L.H. Gross* are used medicinally in various regions of Bangladesh, Myanmar, India, Thailand, and Vietnam. We are aware that no prior experimental research has been done to describe the thrombolytic activity, antidepressant activity, and anxiolytic activity properties of *Persicaria chinensis*. The purpose of the study was to examine the thrombolytic, antidepressant, and anxiolytic activity of the Methanol extract of *Persicaria chinensis* leaves (MEPCL) in contrast to the standard drug that is commercially used.

Highlights

MEPCL exhibited 15.44% clot lysis, lower than streptokinase (79.42%) but significantly higher than distilled water (4.51%).

MEPCL reduced immobility time in the forced swim test and the tail suspension test, with effects approaching Fluoxetine.

MEPCL increased head dipping and time spent in open arms, demonstrating anxiolytic effects similar to diazepam.

Material and Methods

Herb collection, identification, and extraction

The leaves of the *Persicaria chinensis* herb were collected from the Hazarikhil Wildlife Sanctuary, located in the Ramgarh-Sitakunda forests of Chittagong, Bangladesh. It was identified by a renowned botanist from the University of Chittagong's Department of Botany, and a specimen voucher (Accession No. MA221223-06) was kept there for further examination (Supplementary Figure 1). After bulk collection, the leaves were finely chopped and placed in a dry, open, and shaded area for 20 days. The next step involved using a grinder to reduce the herb to a coarse powder. Then, for 10 days, while sometimes shaking and stirring to aid extraction, 400 grams (g) of powder were steeped in 1,500 milliliter (mL) of methanol in a glass jar. With the help of a Buncher funnel, the materials soaked in methanol were passed through filter paper. The solvent was subsequently evaporated using a water bath at 45 °C. The concentration achieved a reddish brown paste-like state[13].

Drugs and chemicals

Methanol was obtained from the Sigma Chemical Company, St. Louis, Missouri, USA. We acquired Diazepam and fluoxetine hydrochloride, 2 widely used pharmaceuticals, from Square Pharmaceuticals Ltd., Bangladesh. Tween 80 was supplied by BDH Chemicals in Leicestershire, UK. This study only used analytical-grade materials and reagents.

Phytochemical investigation

In order to investigate the secondary metabolites of MEPCL and identify flavonoids, alkaloids, quinones, glycosides, steroids, tannins, phenols, terpenoids, and other phytochemicals, this standard method was used Supplementary Figure 2)[14].

Assessment of saponins: after diluting the MEPCL stock solution (1 mL) with 20 mL of distilled water, the test container was manually shaken for 15 minutes. The presence of saponins was confirmed by forming a froth layer on the test tube's surface.

Detection of carboxylic acid: approximately 2–3 drops of sodium bicarbonate were introduced to 1 mL of MEPCL;

effervescence (bubbling resulting from the release of CO_2 gas) signified the presence of carboxylic acid groups in the sample.

Steroid assay: following the dissolution of the crude MEPCL (0.5 g) in chloroform (10 mL), 1 mL of concentrated H_2SO_4 was added to the test container through the wall sides. The uppermost layer assumed a red hue, while the H_2SO_4 layer exhibited a yellow coloration accompanied by green fluorescence. This suggested that steroids were present.

Glycoside assay: a 5-mL stock solution of MEPCL was treated with 2 mL of glacial acetic acid, adding one drop of concentrated H_2SO_4 on $FeCl_3$. An interface surrounded by a brown ring suggested the existence of glycosides.

Phenols test: a few droplets of $FeCl_3$ were subsequently added to a small quantity of MEPCL that had been treated with 1 mL of distilled water. The presence of phenols was denoted by the development of a dark blue or black hue.

Assay for tannins: after diluting the 3-mL stock solution of MEPCL with chloroform, 1 mL of acetic anhydride was added. H_2SO_4 was subsequently added to the test container via the wall sides. The formation of a green hue indicated the identification of tannins.

Terpenoids assay: a 0.5-mL stock solution of MEPCL was subjected to a layer-forming procedure involving 2 mL of chloroform and 3 mL of concentrated H_2SO_4. The formation of a reddish-brown hue conducted the identification of terpenoids.

Detection of carbohydrates: 2 mL of the extract was combined with 2 drops of Molisch's reagent, followed by the cautious introduction of 1 mL of concentrated sulphuric acid (H_2SO_4) regarding the inner surface of the test tube. The formation of a purple ring at the interface indicated the existence of carbohydrates in the sample.

Evaluation of flavonoids: this test was conducted by dipping a few droplets of diluted sodium hydroxide (NaOH) solution into the stock solution of MEPCL (1 mL). The crude extract, which contained flavonoids, exhibited a profound yellow hue that disappeared when a few droplets of diluted sulfuric acid (H_2SO_4) were added.

Alkali detection using Wagner's reagent test: a few droplets of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 mL of distilled water) were added to a stock solution of MEPCL (1 mL), and the solution was examined for the appearance of a reddish-brown precipitate.

In vitro thrombolytic activity

We used the method described by Prasad et al. in 2007[6] to test whether the MEPCL could break up blood clots through *in vitro* assay. To make a thrombolytic evaluation stock solution, 5 mL of commercially available streptokinase was mixed with 5 mL of sterile water. Participants confirmed no health problems and had not taken any medication in the previous 7 days. Eighteen unique sterile Eppendorf tubes were used to collect 3 mL of venous blood from each volunteer with consent. The tubes were labeled A1, A2, and A3. After incubating the tubes at 37 °C for 45 minutes, serum was extracted from the patient without the clot rupturing. The process persisted following the formation of a clot. We took the weight of each tube while holding it again to estimate how much the clot weighed. The mass of the empty tube divides the mass of the clot that has formed inside the tube. Each Eppendorf tube containing a clot received an addition of 0.1 mL of MEPCL. Streptokinase was introduced into the positive control tube, whereas distilled water was incorporated into the negative control tube. After that, the tubes were kept at 37 °C for half an hour to allow the clot lysis to occur. The removal of liquid from the supernatant post-incubation facilitated the measurement of weight change following the dislodgment of the clot. The following equation was used to calculate clot lysis percentage[15]:

% of clot lysis=(weight of clot after lysis ÷ weight of clot before lysis)x100.

Experimental animals

At the International Centre for Diarrheal Disease and Research in Bangladesh (ICDDR, B), we obtained male *Swiss albino* mice for our study. Around 22-30 g was the weight of these mice at 6 weeks of age. Standard laboratory settings were employed to house the animals, which included a 12-hour natural cycle of day and night, a temperature (23±2 °C) and a relative humidity (55-60%). They could also freely consume commercial pellet feed and water. A 48-hour acclimation period was given to the mice before the commencement of the experiment(16). The ethical approval committee of the International Islamic University Chittagong issued its approval for the study protocol

Acute toxicity study

An acute toxicity assessment was performed to evaluate the potential toxicity and establish a safe dosage of *Persicaria chinensis* extract, adhering to a standard protocol with minor modifications[17]. *Persicaria chinensis* leaves were delivered orally at dosages of 1,000 and 1,500 milligram per kilogram (mg/kg) in a 1% Tween solution, while the control group received only the vehicle. Indicators of toxicity or aberrant behaviour were examined following the initial 24 hours and after 4 days of continuous monitoring.

In vivo study design

We established 4 groups of experimental animals, each including 3 mice (n=3): the first group for the control, the second for the standard, and the other 2 groups for testing purposes (MEPCL-200 mg/kg and MEPCL-400 mg/kg). Mice experienced EPM and HBT tests following administration of the standard medication diazepam (1 mg/kg, body weight (b.w.), Intraperitoneal (i.p.)). Mice performed the tail suspension test (TST) and forced swimming test (FST) with the administration of fluoxetine HCl at a dosage of 20 mg/kg body weight. One group received a vehicle consisting of 1% Tween 80 in distilled water at a dosage of 10 mL/kg, administered orally, while the other 2 groups received oral MEPCL at doses of 200 and 400 mg/kg (based on the acute toxicity test and recent literature review[13]), respectively. We administered the standard medications, fluoxetine HCl and diazepam, 15 minutes before the investigations. Both doses of MEPCL or the vehicle were given half an hour before the test.

Antidepressant activity

Forced swim test

We investigated MEPCL's antidepressant effects in Swiss albino mice using the FST method[13]. The animals used for the experiments were placed in open-topped cylindrical containers measuring 25 cm in diameter and 10 cm in height. Water in the container was observed to have a temperature of 25±1 °C. A camera was used throughout the six-minute test; the first 2 minutes were for adaptation, and the latter 4 minutes were for total immobility. As detailed in Section 2.4, the study design was followed for treating the 4 groups of mice, all observed while immobile.

Tail suspension test

To determine antidepressant action, the TST is likely the most straightforward and most reliable approach. The previously mentioned method has been applied to quantify the total duration of TST-induced immobility[13]. Each mouse was suspended 50 cm above the floor by tape, with its tail tip hanging approximately 1 centimeter (cm) below. A camera was used to record the test for 6 minutes. The first 2 minutes were devoted to adaptation, while the last 4 minutes represented the complete immobility time. The study design indicated the administration of treatments to the 4 groups of mice.

Anxiolytic activity

Hole-board test

Utilizing a box measuring 20 cm by 40 cm and with a bottom set 25 cm above ground and 16 holes uniformly placed at a height of 15 cm and 10 centimeters between each hole, the hole-board instrument was employed. The experimental animals were given test doses and left to walk free for 30 minutes after being placed in the middle of the box[13]. Ultimately, a camera captured the mice's heads dipping numerically through the holes for 5 out of the 6 minutes. The first minute was used for adaptation. If the test animals' eyes fell into the hole, it was considered a head drop. Following the protocol established in Section 2.4, treatments were given to the 4 groups.

Elevated plus maze test

The EPM comprised 35 by 5 cm of 2 open arms and 35 by 5 by 20 cm of 2 closed arms; 5 cm × 5 cm of center square was used to join the arms. The device was hoisted to a 25-cm height above the floor. The middle platform, shaped like a plus sign, was formed by joining 2 arms. With their heads turned toward the open arms, the animals were placed in the middle of the EPM apparatus after 30 minutes of dosing[13]. The mouse's behavioral effects were observed using a camera for 5 of the 6 minutes. The first minute served as an initial adjustment period for 2 distinct parameters: the frequency of entries in the open arms and the duration spent in the open arms. The study design was followed to administer treatment to all 4 groups of mice.

Statistical analysis

Data were calculated using Microsoft Excel version 11. Subsequently, Dunnett's test was implemented, followed by a one-way analysis of variance (ANOVA). GraphPad Prism Version 8.0, which was made by GraphPad Software Inc. in San Diego, California, was used for this. The study's statistical differences were examined by comparing all test groups with control groups using the data collected and shown as mean±standard error (SEM). A p-value below 0.05 was considered statistically significant.

Results

Phytochemical screening

A qualitative phytoconstituents investigation was performed to determine the various phytochemicals present in MEPCL. Table 1 shows the main phytoconstituents identified by the current study.

Table 1 Qualitative phytochemical analysis of Methanol extract of *Persicaria chinensis* leaves (MEPCL)

Phytochemicals	Appearance	Observation
Detection of Saponins	Foam formation	+
Detection of Carboxylic acid	Effervescent	-
Detection of Steroids	The upper layer turned red, layer showed yellow with green fluorescence	+
Detection of Glycosides	Brown ring	+
Detection of Phenolic compounds	Deep blue/black color	+
Detection of Tannins	green color	+
Detection of Terpenoids	reddish-brown color layer	+
Detection of Quinones	Red ppt	+
Detection of Carbohydrates	Purple ring	+
Detection of Flavonoids	Yellow ppt	+
Detection of Alkaloids	A reddish-brown color	+

"+" represants the presence of phytochemical while "-" represants absance

In vitro thrombolytic activity

Figure 1 shows the percentage of thrombolytic activity of crude MEPCL. The extracts exhibited significant clot lysis activity. As positive and negative controls, streptokinase (79.42%, p-value<0.0001) and distilled water (4.51%), respectively, demonstrated clot lysis. The percentage of thrombolytic activity is used to describe the reduction in clot weight. The clot lysis activity of MEPCL was 15.441% (p-value=0.0141).

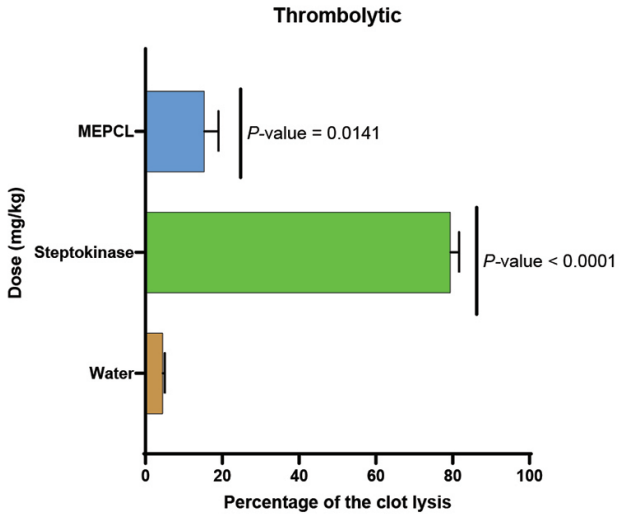

Figure 1 Thrombolytic activity of Methanol extract of *Persicaria chinensis* leaves (MEPCL) and Streptokinase (Positive control) compared with distilled water (Negative control). The outcomes have been manifested as mean±standard error (SEM) (n=3) with the statistically significant (F (2, 15)=264.2; p-value<0.0001) in comparison to the control by the Dunnett test.

In vivo antidepressant activity

Animals tested in this experiment showed shorter immobility durations after receiving 2 doses of MEPCL, as shown in the current study. Results showing Fluoxetine as a standard, extract, and control with 1% Tween 80 are shown in Figure 2. The immobility duration of both dosages of the MEPCL-treated groups was significantly reduced (155 ±4.01 sec and 141±8.50 sec, respectively). However, it was minor compared to the Fluoxetine 20 mg/kg, which showed a decrease in immobility time of 82±3.055 sec (F (3, 8)=165.1; p-value<0.0001). With values of 138.67±3.48 (p-value=0.0303) and 126.33±1.45 (p-value=0.0040) respectively, the results showed that the mice's immobility times in the forced swim test (Figure 3) were significantly shorter than those in the positive control group, which had an immobility time of 82.00±2.0, which was also considerably different (F (3, 8)=53.38; p-value<0.0001).

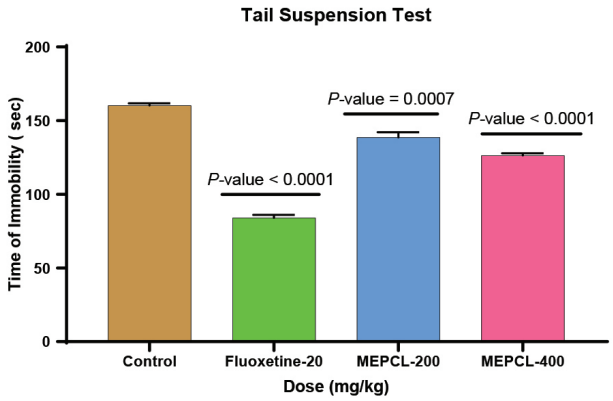

Figure 2 Antidepressant activity of Methanol extract of *Persicaria chinensis* leaves (MEPCL) and Fluoxetine compared with control in tail suspension test on mice. The outcomes have been manifested as mean±standard error (SEM) (n=3) with statistically significant (F (3, 8)=165.1; p-value<0.0001) in comparison to the negatived control group processed by the Dunnett test.

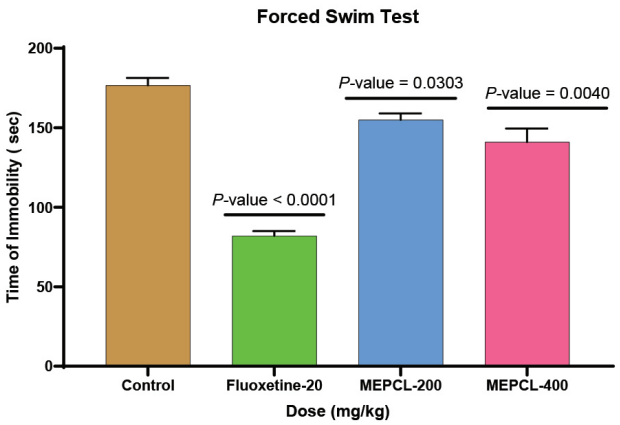

Figure 3 Antidepressant activity (immobility time) of Methanol extract of *Persicaria chinensis* leaves (MEPCL) and fluoxetine in forced swimming test. The outcomes have been manifestedas mean± standard error (SEM) (n=3) with a statistically significant (F (3, 8)=53.38; p-value<0.0001) in comparison to the negative control group processed by the Dunnett test.

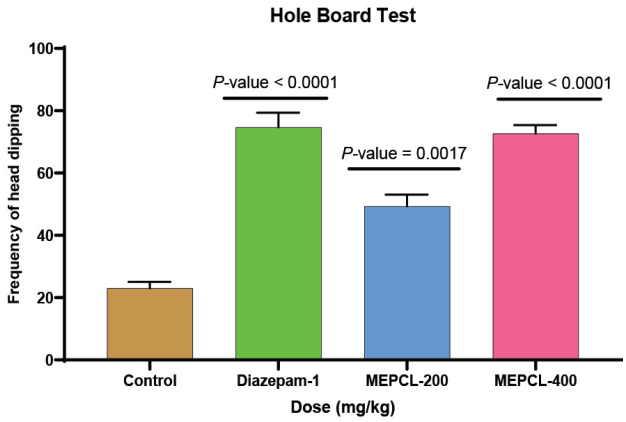

Figure 4 Anxiolytic activity (number of head dipping) of Methanol extract of *Persicaria chinensis* leaves (MEPCL) and diazepam in Hole board test. The outcomes have been manifested as mean± standard error (SEM) (n=3) with the statistically significant (F (3, 8)=49.09; p-value<0.0001) in comparison to the control group processed by the Dunnett test.

In vivo anxiolytic activity

Figure 4 shows that the number of head dips underwent a statistically significant decrease after administering MEPCL doses of 200 and 400 (mg/kg), along with the standard drug (diazepam) in the hole-board test, compared to the negative control group. The occurrence of head dipping was considerably increased (49.33± 3.71; p-value=0.0017 for 200 mg/kg and 72.67±2.73; p-value<0.0001 for 400 mg/kg) in the group given the greater dosage, whereas the standard drug demonstrated 74.67±4.70; p-value<0.0001. The findings showed that *Persicaria chinensis* has anxiolytic properties.

In this study, diazepam was used as a standard medication, and it was discovered that MEPCL lengthened the time of stay in open arms. When comparing the control groups, the doses of MEPCL-200 (14.33±0.88, 50±2.65) and MEPCL-400 (19±1.53, 62.66±2.29) and diazepam (27.67±3.18, 77.89±2.60) showed an increase in the frequency of entries into the open arm and time spent in the open arm (Figures 5A and 5B). The amount of time spent in the open arm at the high dose of MEPCL was more significant compared to the lower dose.

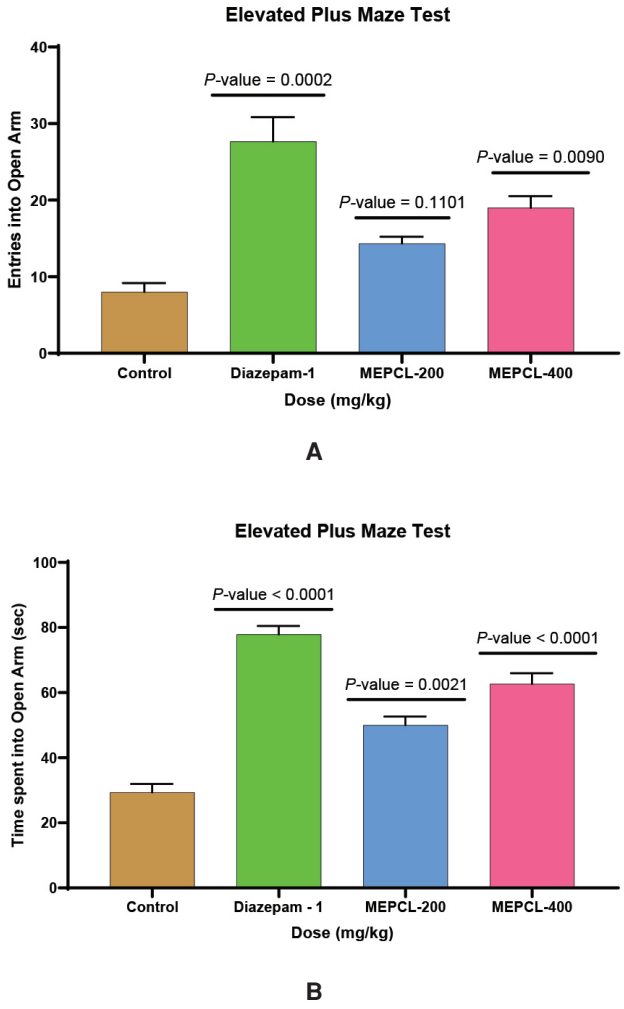

Figure 5 Anxiolytic activity (number of entries into open arm (A) and time spent into open arm (B)) of Methanol extract of *Persicaria chinensis* leaves (MEPCL) and diazepam in Hole board test. The outcomes have been manifested as mean±standard error (SEM) (n=3) with the statistically significant ((F(3, 8)= 18.84; p-value=0.0006) in comparison to the control group processed by the Dunnett test.

Discussion

Thrombosis, anxiety, and depression have all been treated with numerous therapies; however, the most effective method for reducing disease symptoms without causing negative effects remains unknown. Consequently, the practical application of multiple medications is hampered by their adverse effects and insufficient pharmacokinetics. Thus, there is a rising demand for innovative medicines and a growing concern regarding the efficacy, safety, and adverse effects of current medications.

A new and crucial function of plant chemicals has evolved in light of their purported immunomodulatory effects, prompting extensive scientific investigation to establish safety and effectiveness[18,19]. Studies have shown that dietary supplements derived from various plant sources can reduce the risk of cardiovascular difficulties and stroke by acting as anticoagulants, anti-platelets, and fibrinolytics[20,21]. Several thrombolytic medicines have been sourced from different places. It is possible to prevent thrombus development by using antithrombotic or thrombolytic medications[22]. Thrombolytic therapy relies on plasmin, which can be triggered from dormant plasminogen, to break down fibrin[23]. Staphylokinase breaks down cell-binding fibrin fibers while activating plasminogen to dissolve clots[24]. The main goal of this thrombolytic assay was to see how well the extracts can break up blood clots that are already there. Blood clots can cause serious health problems that can be fatal, like pulmonary embolism, ischemic strokes, myocardial infarction, and so on[5]. In our study, the extracts exhibited significant clot lysis activity. As positive and negative controls, streptokinase (79.42%, p-value<0.0001) and distilled water (4.51%), respectively, demonstrated clot lysis. The clot lysis activity of MEPCL was 15.441% (p-value=0.0141). The results showed less potential to lyse the clot after treating them with the MEPCL, as evidenced by the substantial thrombolytic activity found after treatment compared to the negative control. This finding could potentially impact cardiovascular health, particularly for those with atherothrombosis. This is just a preliminary trial, and more research may be required to reach definitive results regarding these herbs' potential as thrombolytic

medications. Research should be conducted to determine the precise mechanism of action and the molecular structure of the active components found in many plant extracts.

The FST and TST are well-known methods for estimating antidepressant activity. The distinctive behavior assessment, immobility in these investigations, was compared to human depression. Close to the drug's antidepressant potential is its ability to shorten immobility durations in mice. Several chemicals with antidepressant potential were discovered by employing swimming as an objective. Controlled swimming can be done with drugs like opioid receptors, nitric oxide production, glutamate metabotropic receptors, melanocortin-4, GABA (gamma-aminobutyric acid) receptors, and brain-derived neurotrophic factor antagonists. For 5-HT (5-hydroxytryptamine) to have an antidepressant effect on the swimming test, SSRIs (selective serotonin receptor inhibitors) need to be able to keep making it[3]. The presence of phytochemicals in the MEPCL is also thought to increase 5HT transmission, enhancing FST and TST mobility. Animals tested in this experiment showed shorter immobility durations after receiving 2 doses of MEPCL (200 and 400 mg/kg). The immobility duration of the 2 MEPCL groups was significantly reduced (155±4.01 sec and 141±8.50 sec, respectively). However, it was minor compared to the positive control group (Fluoxetine 20 mg/kg), which showed a decrease in immobility time of 82±3.055 sec ($F(3, 8)=165.1$; p-value<0.0001). With values of 138.67±3.48 (p-value=0.0303) and 126.33±1.45 (p=0.0040) respectively, the results showed that the mice's immobility times in the forced swim test (shown in Figure 3) were significantly shorter than those in the negative control group, which had an immobility time of 82.00± 2.0, which was also considerably different ($F(3, 8)=53.38$; p-value<0.0001).

The anxiolytic effects of plants with alkaloids, flavonoids, terpenes, or saponins were demonstrated in earlier research[25,26]. Traditional medicine uses a wide variety of soothing herbs because flavonoids act on central benzodiazepine receptors in the central nervous system[27]. The anxiolytic effects of flavonoids are due to their interaction with GABA receptors. Even at low concentrations, they can regulate GABAA receptors, regardless of whether or not they are sensitive to flumazenil[28]. Hence, they can influence GABAergic system receptors through and independent of the traditional benzodiazepine-binding site. In previous research, the GABA-induced chloride current (IGABA) was found to be highly regulated by apigenin and diazepam[29]. There is a direct correlation between animals' emotional states and the head-dipping habit[30]. The frequency with which animals dip their heads is correlated with their level of head-dipping behavior[31]. Figure 4 shows that there was a statistically significant increase in head dipping after administering MEPCL doses of 200 and 400 mg/kg along with the standard drug (diazepam) in the hole-board test, as compared to the control group. The findings showed that *Persicaria chinensis* has anxiolytic properties aligned with the standard drug diazepam.

The EPM test determined the medications' anxiolytic effects[32]. In EPM, increasing the frequency of entries into the open arm and time spent in the open arm strongly correlate with anxiolytic behavior[33,34]. In this study, it was revealed that MEPCL lengthened the time of stay in open arms. When comparing the experimental group with the control group, the doses of MEPCL-200 (14.33±0.88, 50±2.65) and MEPCL-400 (19±1.53, 62.66±2.29) and diazepam (27.67±3.18, 77.89±2.60) showed an increase in the number of entries into the open arm and time spent in the open arm (Figures 5A and 5B), where the amount of time spent in the open arm at a high dose of MEPCL was more significant compared to a lower dose, which suggested that MEPCL has strong anxiolytic activity.

Study limitation

A notable constraint of our work is the limited sample size employed in the *in vivo* studies, which may compromise the generalizability of the findings. Moreover, despite implementing standardized protocols and blinding to mitigate experimental bias, nuanced and contextual handling of the variables may still have affected behavioral assessments like the Tail Suspension Test, Forced Swim Test, Hole Board Test, and Elevated Plus Maze. The lack of sophisticated analytical methods, such as LC-MS for component identification, constrains the thoroughness of phytochemical characterization. These must be considered when interpreting the findings, and further research is necessary in order to validate and enhance these results by using bigger, more diversified animal populations and thorough analytical techniques.

Conclusion

The study uncovered that *Persicaria chinensis L.H. Gross* leaves exhibited potent antidepressant and anxiolytic effects, although they had only minor thrombolytic activity. This research provided new information about the thrombolytic, antidepressant, and anxiolytic properties of *Persicaria chinensis L.H. Gross* leaves. Even though MEPCL has low thrombolytic activity, it may be a helpful treatment for cardiovascular health and help avoid small thrombotic episodes. MEPCL's multi-target effects make it a viable option for integrative treatment strategies that address minor circulatory and mental health issues. The current findings highlight its therapeutic adaptability as a plant-based intervention. However, large-scale investigations are required to assess the herb's safety in treating thrombosis and central nervous system disorders. Furthermore, additional research is required in the future to support the extended therapeutic use of under-observed herbs in order to support their dose-related activity, which is tolerable for the growth of normal cells.

Author's contribution

Conceptualization, M.R.K.; methodology, M.A.; software, R.R.; investigation, M.A.; resources, T.J.; data curation, R.R.; formal analysis and visualization, R.R.; writing—original draft preparation, R.R. and T.J.; writing—review and editing, R.R., M.A.S., and M.R.K.; supervision, M.R.K. All authors have read and agreed to the published version of the manuscript.

Source of the plant

This plant was collected from the hill tracks of Hazarikhil Wildlife Sanctuary, Ramgarh-Sitakunda forests, Chattogram, Bangladesh, which was identified by a renowned botanist from the Department of Botany at the University of Chittagong. A specimen voucher with Accession No. "MA221223-06" has been preserved at that site for future inspection (Supplementary Figure 1).

Ethical approval

The relevant committee of the Department of Pharmacy approved all of the trials, which were conducted under NIH criteria (Ref: Pharm/P&D/241/17-'23)

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Conflict of interest

The authors declare that they had no competing interests.

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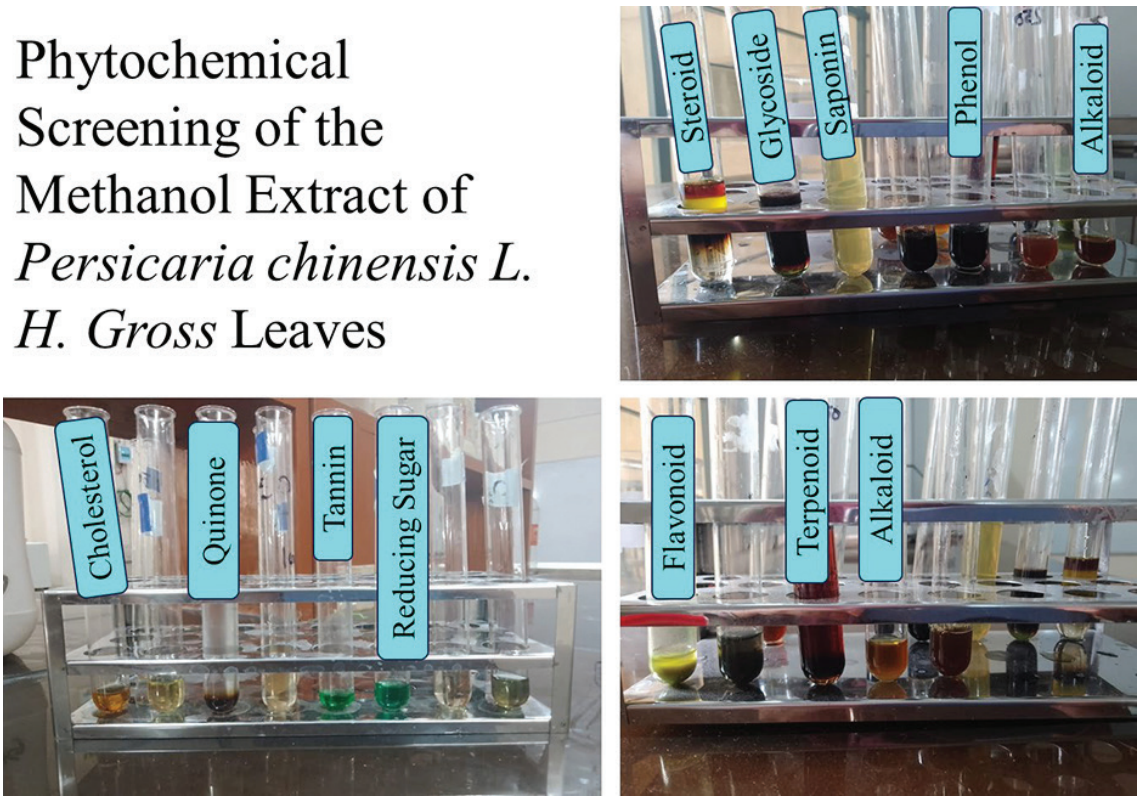
Supplementary Figure 1 Herbarium sheet



Supplementary Figure 2 Phytochemical screening