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IN VITRO AND *IN VIVO* PHARMACOLOGICAL EVALUATION OF *POLYGONUM PERSICARIA* L.

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Abstract

The present study was designed to investigate the *in vitro* antioxidant, cytotoxic, anthelmintic and *in vivo* analgesic, anti-diarrheal, hypoglycemic and CNS anti-depressant properties of different solvent fractions of methanol extract of leaf and flower of *Polygonum persicaria* L., a notable medicinal plant in Bangladesh. The crude methanol extracts were partitioned separately to petroleum-ether, carbon tetrachloride, chloroform and aqueous fractions. In DPPH scavenging assay for antioxidant test, the aqueous fraction of flower extract showed maximum antioxidant activity with IC_{50} of 0.60 µg/ml. Most of the fractions revealed prominent lethality (LC_{50}) against brine shrimp nauplii. The test samples also exhibited dose-dependent anthelmintic activity against *Pheretima posthuma*. The leaf extract and flower extract (200-and 400 mg/kg b.w.) can decrease the painful sensation in mice. Leaf extracts and flower extracts displayed 57.69 and 50% reduction of diarrhea induced by castor oil in mice, respectively. The plant samples exhibited hypoglycemic effect and CNS anti-depressant effect in mice. Based on the possibility of bioactivity, the plant can be thoroughly examined to determine its potential efficacy and to support its traditional uses.

Introduction

Plants generate a variety of chemical compounds in order to accommodate their solitary and phototrophic lifestyle that help them adapt to changes in their surroundings and the phototropic cycle. Over an eternity ago, humans have known that certain plants are therapeutically useful for specific ailments (Abdallah *et al*. 2023). Many of the therapeutic compounds used in contemporary medicine have their origins in plant-based substances and around 14-28% of higher plant species are thought to have therapeutic uses (Newman and Cragg 2016). They have been discovered as a result of the evidence-based approach to drug development and the key pharmacophores have been modified to improve their pharmacological, pharmacokinetic and physicochemical qualities against a range of disorders (Atanasov *et al*. 2021). As the demand for herbal pharmaceuticals is growing globally, there is a clear increase in scientific and commercial interests in using medicinal plants as raw materials (Rahman *et al*. 2022).

There are 250 species of both annual and perennial herbs in the genus *Polygonum* belonging to Polygonaceae Family. Since there are differences in their morphology and phytochemistry, these species have been classified into the *Persicaria* and *Polygonum* genera (Seimandi *et al*. 2021). Some biological qualities of *Persicaria* and *Polygonum* species include antifungal (Lopez *et al*. 2011), analgesic, antiseptic, diuretic, antirheumatic, astringent etc.

Polygonum persicaria L. belonging to family Polygonaceae is native to Europe, but it is a common weed throughout the temperate and tropical regions of Asia, North and South America, North Africa and Australia. *P. persicaria* is a perennial plant with incredibly diverse morphology. The plant flourishes in moist environments like wetlands and the banks of the river and typically

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predominates in the fields of agriculture. This species contains phenolic acids, chalcones, flavanones, stilbenes and flavones, according to earlier phytochemical research. There are numerous typical medical uses for *P. persicaria*. The herb has been used in Europe to treat gynecological diseases as well as emetic and diuretic. A decoction of the whole herb is used to cure dyspepsia, itchy scalp, diarrhea, hemorrhoids and frequent menstrual bleeding. Seeds and leaves are used to treat cancerous growth in individuals. An extensive review of the literature reveals that very little scientific research on Bangladeshi origin *P. persicaria's* biology and pharmacological studies have been produced to validate its therapeutic benefits. Therefore, as a part of the ongoing studies with Bangladeshi medicinal plants (Islam *et al*. 2019, Sultana *et al*. 2022, Rakhi *et al*. 2024) *P. persicaria* was carefully investigated in this study in order to assess its *in vitro* and *in vivo* pharmacological potentials using appropriate experimental models.

Materials and Methods

Morphine, diclofenac sodium, loperamide, glibenclamide and phenobarbitone sodium were collected as gift from Square Pharmaceuticals Ltd, Beximco Pharmaceuticals Ltd, Bangladesh and Incepta Pharmaceuticals Ltd, Bangladesh. Chemicals and reagents such as gallic acid, butylated hydroxytoluene (BHT), ascorbic acid (AA), dimethylsulphoxide (DMSO), vincristine sulfate (VS), acetic acid and Tween 80 were of analytical grade and purchased from local suppliers.

Leaves and flowers of *P. persicaria* were collected from Sonargaon in Narayanganj, Dhaka, Bangladesh. The plant was identified taxonomically by a Senior Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka. Leaves and flowers were thoroughly cleaned and allowed to air dry for several days. After drying, the plant samples were ground to make a course powder.

The leaf and flower powders, total around 650 g was separately soaked in methanol for several days while being shaken and stirred periodically in order to prepare the crude extract. Subsequently, the blend was passed through Whatman filter paper number 1. The leaf powder filtrate was then concentrated using a Buchi rotary evaporator at 40°C and low pressure, yielding a semisolid mass that was also known as *P. persicaria*'s crude methanol extract of leaf (LME). Similarly, the crude methanol extract of flower (FME) of *P. persicaria* was obtained by the above mentioned extraction process. Both crude extracts (each about 5 g) were subjected to modified Kupchan method (VanWagenen *et al*. 1993) to get the different fractions (Table 1).

Table 1. Test samples of leaves and flowers for biological investigation.

Plant part	Sample code	Test samples of *P. persicaria*
Leaves	LME	Methanolic extract of leaf
	LPE	Petroleum-ether partitionate of leaf
	LCTC	Carbon tetrachloride partitionate of leaf
	LCH	Chloroform partitionate of leaf
	LAQ	Aqueous soluble partitionate of leaf
Flowers	FME	Methanolic extract of flower
	FPE	Petroleum-ether partitionate of flower
	FCTC	Carbon tetrachloride partitionate of flower
	FCH	Chloroform partitionate of flower
	FAQ	Aqueous soluble partitionate of flower

A spectrophotometric technique (Harbertson and Spayd 2006) involving Folin-Ciocalteau was used to determine the total phenolic content of the plant sample using gallic acid as the standard. Briefly, about 0.5 ml of plant extract (2 mg/ml) was added to the mixture of 2.5 ml of Folin-Ciocalteau reagent (10 times diluted) and 2.0 ml of 7.5% Na_2CO_3 solution. The reaction mixture was left for 20 min in dark. At 760 nm, the absorbance was measured with a spectrophotometer. By utilizing the findings from the gallic acid solution to create a calibration curve, the total phenolic content of the plant extracts was determined. Gallic acid equivalent (GAE) in milligram (mg) per gram (gm) of the plant extract was used to express the results.

Antioxidant activity was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (Brand-Williams *et al.* 1995) of the plant samples. In short, about 2.0 ml of each diluted plant extract solution with varying conc. from 0.977 to 500 µg/ml was added to 3.0 ml of DPPH working solution (20 µg/ml conc.). Then the mixture was incubated at room temperature without illumination for 30 min. Following that, a UV-Vis spectrophotometer (Shimadzu, Japan) was used to measure the absorbance at 517 nm. As reference standards, butylated hydroxytoluene and ascorbic acid were applied.

$$\% \text{ inhibition of DPPH scavenging} = \frac{A_{DPPH} - A_{Sample}}{A_{DPPH}} \times 100\%$$

Where, A = absorbance of the respective test sample.

The cytotoxic activity was evaluated by brine shrimp lethality bioassay (Meyer *et al.* 1982). Here, brine shrimp eggs were raised in simulated sea water for the purpose of producing nauplii. The desired concentration of the test samples was created by adding the specified amount of DMSO. Vincristine sulfate (VS) was used as positive control.

In vitro anthelmintic properties of all solvent extracts of leaf and flower were evaluated on *Pheretima posthuma* (Annelida) at three different concentrations of 5, 10 and 20 mg/ml, respectively, with albendazole acting as the standard and saline water as the negative control (Das *et al.* 2011).

The *in vivo* pharmacological study was conducted with Swiss-albino mice of either sex, aged 4-5 weeks that were purchased from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Animals were housed in a polypropylene cage with adequate ventilation and maintained at a controlled temperature of 24 ± 2°C with a relative humidity of 60-70% throughout 12 hrs of light and dark cycles. The mice were given regular commercial diets from the suppliers along with water *ad libitum.* Prior to and throughout the trial, food was withheld for 12 hrs (Zimmermann 1983).

During each *in vivo* experiment, a total of 18 animals were chosen at random and split up into six groups, each consisting of three mice: Groups I, II, III, IV, V, and VI. Different treatments were given to each animal group. Each mouse was accurately weighed prior to the experiment, and the doses of the test samples and control materials were modified correspondingly. Since it was challenging to watch three mice getting the same therapy at once, it was important to identify each animal in the group as it was receiving treatment.

The central analgesic efficacy of the plant extracts was assessed using the tail immersion procedure (Islam *et al.* 2019). Three mice in each group were assigned to the negative control group, the positive control group and the plant samples group. The negative control group and test groups received 1% Tween 80 in normal saline and the test materials of *P. persicaria* (200-and 400 mg/kg b.w.) orally while the positive control group was given standard morphine solution (2 mg/ml) subcutaneously. In order to conduct the test, a water bath with warm water kept at a steady 55°C was used to submerge 1-2 cm of the mouse's tail. Timed tail deflections were taken of the

mice before the treatments (0 min), and then again 0, 30, 60, and 90 min later. The following formula was used to determine the percent (%) time elongation relative to the control mice:

$$\% \text{ Time elongation} = \frac{T_{Test} - T_{Control}}{T_{Control}} \times 100\%$$

Here, T = average time of tail deflection in the respective group.

Acetic acid-induced writhing method (Koster *et al.* 1959) was utilized to assess the effectiveness of plant samples as peripheral analgesics. Acetic acid solution was subcutaneously injected to the right hind paws of the experimental animal to induce the abdominal writhes. The mice were given test samples orally for 45 min, and then 10 ml/kg of 0.6% (v/v) acetic acid was injected subcutaneously to trigger writhing. For every group of mice, the number of writhes (abdominal constrictions) was counted beginning 5 min after the formalin injection and continuing for 10 min. Using the following formula, the percentage inhibition of writhing was obtained:

$$\% \text{ Inhibition of writhing} = \frac{N_{Control} - N_{Test}}{N_{Control}} \times 100\%$$

Here, N = mean number of writhing in respective group.

Anti-diarrheal efficacy was evaluated by inducing diarrhea in mice using castor oil (Shoba and Thomas 2001). A standard dose of loperamide (10 mg/kg b.w.) was administered to the positive control group, and 10 ml/kg b.w. of 1% Tween 80 in normal saline was given to the negative control group. The test groups were given both leaf and flower extract at the doses of 200- and 400 mg/kg b.w. The plant extract was found to have a percentage reduction in diarrhea based on the number of diarrheal feces emitted by the mice, which was monitored for up to 4h of experiment. The percentages (%) reduction in diarrhea was determined using the following formula:

$$\% \text{ Reduction of diarrhea} = \frac{D_{Control} - D_{Test}}{D_{Control}} \times 100\%$$

D = number of diarrheas in respective group.

During hypoglycemic activity test (Mursalin *et al.* 2023), oral doses of standard glibenclamide (10 mg/kg b.w.) and plant samples (200-and 400 mg/kg b.w.) were administered to the mice. A 10% glucose solution (2 gm/kg of b.w.) was administered to each group after an hour. Blood was obtained from the mice's tail veins at 1, 2, and 3 hrs after the glucose was administered and blood glucose level was determined by a glucometer.

Phenobarbitone sodium-induced sleeping time test (Dandiya and Cullumbine 1959) was used to assess the CNS anti-depressant efficacy of *P. persicaria*. Here, the negative - and positive-control groups received 1% Tween 80 in normal saline and phenobarbitone sodium (30 mg/kg b.w., i.p.), respectively while the test groups were given the leaf extract and flower extract (200- and 400 mg/kg b.w.) orally. Each mouse in the test groups received an intraperitoneal injection of phenobarbitone sodium (25 mg/kg b.w.) around half an hour later in order to induce sleep. For both the control and treatment groups, the time at which each group fell asleep and stayed asleep were noted.

Results and Discussion

Medicinal plants are unique sources of bioactive compounds with diverse pharmacological actions. Several active constituents have been isolated from them for direct use as therapeutic agents or act as lead compounds (Newman and Cragg 2016). The goal of this study was to assess

the *in vitro* antioxidant, cytotoxic, anthelmintic and *in vivo* analgesic, anti-diarrheal, hypoglycemic, CNS anti-depressant properties of *P. persicaria* leaf and flower extracts.

Phenolic compounds fight against free radicals and confirm protective effects against oxidative stress that is linked to various diseases. The plant products such as vegetables and dietary fruits are rich in phenolic compounds that act as antioxidant which can play a significant benefit in health by reducing oxidative stress. The level of phenolic compounds is correlated with the anti-inflammatory and antioxidant activities of medicinal plants. Total phenolic content analysis of both the methanol extract of leaves and flowers of *P. persicaria* and different solvent partitionates were determined by Folin-Ciocâlteu colorimetric method. In this ionized phenolic solution, the Folin-Ciocalteu reagent quickly oxidizes the phenols. After the oxidation process, yellow Folin-Ciocalteu reagent becomes blue. With a spectrophotometer set at 760 nm, the level of color change is measured. The assay evaluated the samples' total phenolic content, which was reported as mg of gallic acid equivalent (GAE)/g of extractives. For leaves, the amount of total phenolic content differs in different extractives and ranged from 0.96 to 6.20 mg of GAE/gm of extractives (Table 2). Out of all the leaf extractives, LCTC had the highest phenolic concentration (6.20 mg of GAE/g of extractives). In case of flowers, the amount of total phenolic content differs in different extractives and ranged from 5.03 to 10.28 mg of GAE/g of extractives of *P. persicaria*. Among all extractives, the highest phenolic content was found in FME (10.28 mg of GAE/g of extractives) followed by FAQ (7.56 mg of GAE/g of extractives).

The antioxidant activity was assessed by DPPH radical scavenging assay using BHT and ascorbic acid as standards. DPPH is a commonly used method to assess the antioxidant activity of phytoconstituents to test a compound's capacity to operate as a hydrogen donor or free radical scavenger. The antioxidant potential was expressed as IC_{50} value. In the present study, the solvent fractions of flower were found to be more powerful antioxidant than that of leaf. Among all, both the aqueous fractions FAQ and LAQ showed significant antioxidant potential with IC_{50} value of 0.60 and 14.60 µg/ml, respectively (Table 2). At the same time LCTC, LCH and LME also exhibited antioxidant potential having IC_{50} values of 17.65, 20.81and 24.20 µg/ml, respectively. Compared to standard AA (IC_{50} = 5.8 µg/ml) and BHT (IC_{50} = 27.5 µg/ml), all the solvent fractions obtained from flower part demonstrated strong antioxidant potential with IC_{50} values ranging from 0.60-6.53 µg/ml. In a previous study of Dar *et al*. (2022), the aqueous extract of *P. persicaria* at a concentration of 400 µg/ml exhibited 53.63% DPPH radical scavenging activity. Phenolic components, including flavonoids, phenolic acids, and phenolic diterpenes, are primarily responsible for the anti-oxidative impact. By absorbing and neutralizing free radicals, these substances use their antioxidant qualities to safeguard the biological system.

The lethality bioassay method for brine shrimp (Meyer *et al*. 1982) is a quick universal bioassay methodology for natural materials that shows cytotoxicity along with a variety of pharmacological activity such as antiviral, and anticancer properties, among others. The lethality of the extractives to brine shrimp was expressed as LC_{50}. The cytotoxicity of the different extractives was compared with standard vincristine sulfate. In the present investigation, the LC_{50} values of the solvent fractions from leaf part such as LME, LCTC, LPE, LAQ and LCH were found to be 0.54, 0.96, 1.92, 12.97 and 40.01 µg/ml, respectively (Table 2). For flowers, the LC_{50} value of FCTC, FPE, FME and FAQ were found 0.18 µg/ml, 0.53 µg/ml, 4.35 µg/ml and 14.56 µg/ml, respectively which showed significant activity (Table 2). The inhibitory effect of the extract might be the result of the presence of toxic compounds in the extract of *P. persicaria* which warrants further investigation.

Anthelmintic activity of methanol extract of leaves and flowers was determined using *P. posthuma* as test worms. In the present study, the test samples exhibited concentration-dependent

anthelmintic activity as evidenced by decreased paralyzing time and death time, with increasing concentration of the test samples (Table 3). Evaluation of anthelmintic activity was compared with reference standard albendazole. Here, anthelmintic potential of the test samples was expressed as follows: albendazole > methanol extract of leaf, LME > methanol extract of flower, FME. Albendazole (10 mg/ml) shows paralysis at 25 min and death at 55 min where the study sample, LME caused paralysis at 22, 25 and 26 min and death at 53, 66 and 76 min at 20, 10 and 5 mg/ml, respectively (Table 3). The flower extract of *P. persicaria* at the same doses showed paralysis at 22, 34 and 41 min and death at 54, 63 and 80 min at 20, 10 and 5 mg/ml, respectively. Taking into account that the leaf methanol extract exhibited similar action, it would be crucial to determine the primary phytoconstituents.

Table 2. TPC analysis, antioxidant and cytotoxic activities of crude methanol extracts and fractions of leaves and flowers of *P. persicaria*.

Test sample	Antioxidant activity		Cytotoxic activity
	Total phenolic content (mg of GAE/gm of extractives)	DPPH assay IC_{50} (µg/ml)	LC_{50} (mg/ml)
LME	1.39	24.20	0.54
LPE	0.96	60.25	1.92
LCTC	6.20	17.65	0.96
LCH	5.15	20.81	40.01
LAQ	2.38	14.60	12.97
FME	10.28	2.42	14.56
FPE	5.03	6.53	0.53
FCTC	6.70	2.56	0.18
FCH	5.28	1.37	51.22
FAQ	7.56	0.60	4.35
BHT	--	27.5	--
AA	--	5.8	--
VS	--	--	0.45

Table 3. Anthelmintic activity of methanol extracts of leaves and flowers of *P. persicaria*.

Treatment	Concentration (mg/ml)	Paralyzing time (min)	Death time (min)
Saline Water	9	--	--
Albendazole	10	25	55
Leaf extract, LME	20	22	53
	10	25	66
	5	26	76
Flower extract, FME	20	22	54
	10	34	63
	5	41	80

Analgesic chemicals are being searched for in natural sources as potential synthetic medication alternatives due to their natural origin and few side effects. The tail immersion method was applied to assess the analgesic activity of *P. persicaria* extractives, with morphine (2 mg/kg

b.w.) acting as a positive control. This method measures the changes in sensitivity of test animals caused by the analgesic action of medicines. The mice's tail was subjected to continuous heat stress, which triggers discomfort. Mice quickly withdrew their tails when the stimulation level was surpassed. Tail immersion time is the amount of time it takes the mice to remove their tails. Analgesic substances prolong this reaction time. After the drug or extract was administered into the animals, the percent (%) elongation was observed at 30, 60, and 90 min. The percentage of time that the tail immersed was elongated was computed in relation to the control. The group's central analgesic action increased with the group's elongation percentage. In this method, the flower extract at both doses of 200- and 400 mg/kg b.w. showed more analgesic action in mice compared to the leaf extract of *P. persicaria* with same doses. As shown in Fig. 1, the flower- and leaf extract at 400 mg/kg b.w. increased the pain reaction time (PRT) with 213.07 and 132.8% elongation, respectively at 90 min after the oral administration of plant sample in mice which was comparable to standard morphine (167.15%). The flower extract and the leaf extract at the dose of 200 mg/kg b.w. prolonged the PRT with 234.58 and 148.99% elongation at 60 min after that their effects started to diminish at the level of 232.53 and 132.8%, respectively at 90 min after extract administration (Fig. 1). The plant extracts demonstrated a moderately analgesic impact on the experimental mice, according to the results of the tail immersion model.

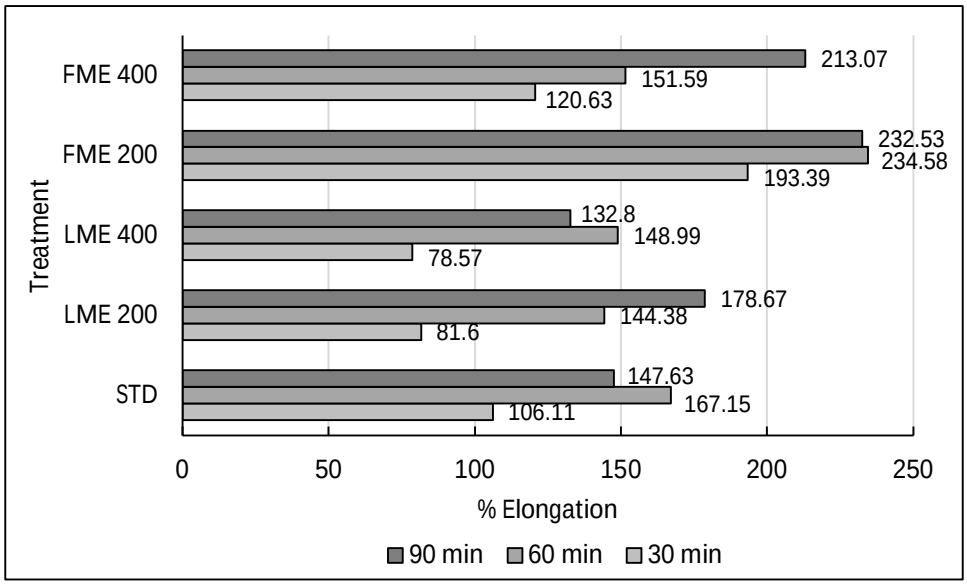

Fig. 1. Central analgesic activity of methanol extracts of leaves and flowers of *P. persicaria*.

Peripheral analgesic activity test was evaluated by acetic acid-induced writhing test in mice model. Here, acetic acid solution was injected subcutaneously into the right hind paw of the experimental animals. Because of this, the animals scream in suffering on a frequent basis. We refer to this movement of the body as "writhing." Every wriggle or contraction reaction was tallied and interpreted as a sign of experiencing discomfort. Any drug with analgesic properties should reduce the number of writhing animals do in a certain amount of time relative to the control group. When compared to the control group, all plant samples in this investigation (200- and 400 mg/kg b.w.) showed a significant dose-dependent decrease in the number of animal writhes in the

abdomen. The standard drug diclofenac sodium inhibits 63.27% writhing at a dose of 25 mg/kg b.w. while the leaf extract and flower extract of *P. persicaria* at the dose of 400 mg/kg exhibited 42.86% and 67.35% inhibition of acetic acid-induced writhing in mice (Fig. 2). Based on the aforementioned findings, it can be deduced that methanolic extract of *P. persicaria* leaf and flower showed a significant analgesic effect in the acetic acid-induced writhing test and tail immersion activity.

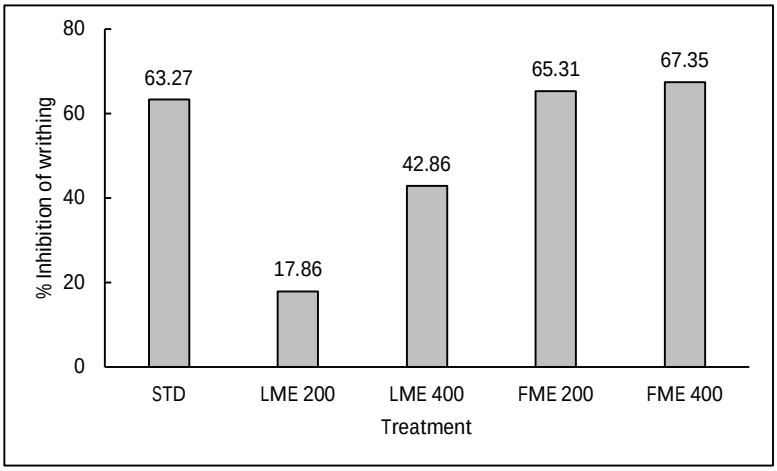

Fig. 2. Peripheral analgesic activity of methanol extracts of leaves and flowers of *P. persicaria.*

The growth and advancement of contemporary research on drugs' anti-diarrheal properties is significantly influenced by medicinal plants. Traditional healers utilize a variety of plants that have anti-diarrheal qualities. Unfortunately, many of these traditional remedies for diarrhea have not had their efficacy well examined by the scientists. The anti-diarrheal property of methanol extract of leaves and flowers was evaluated using castor oil-induced diarrhea in mice. Both the extracts caused a dose-dependent anti-diarrheal effect in mice (Fig. 3). At 4 hrs after diarrhea induction by castor oil, the leaf extract and the flower extract at 400 mg/kg exhibited 57.69% and 50% reduction of diarrhea, respectively in comparison to the standard loperamide (69.23 % reduction of diarrhea). In this test, the leaf extract showed better anti-diarrheal response than that of flower extract in mice. In the treatment of diarrhea, flavonoids, alkaloids, tannins, terpenoids, saponins, and steroids from medicinally active plants serve as vital compounds (Ayalew *et al.* 2022).

Plant-based substances with antidiabetic potential can be utilized as an alternative to synthetic medications due to limitations in the treatment of diabetes. As a result, scientists have been looking for bioactive compounds with the ability to reduce glucose levels in plants. *P. persicaria* leaf and flower extract was administered to the glucose-loaded diabetic mice used in the oral glucose tolerance test. Compared to the control group, all plant extracts (200- and 400 mg/kg) lowered blood glucose levels at experimentation times of 0, 60, 120 and 180 min (Table 4). This impact persisted for up to 120 min following the oral glucose treatment. Plant extracts have the potential to lower plasma glucose levels by many mechanisms, including as inducing β-cells to secrete insulin, blocking pancreatic α-amylase, or expediting the uptake of glucose by peripheral tissues (Mursalin *et al*. 2023). The mechanism underlying these pharmacological activities requires confirmation by additional biological investigations.

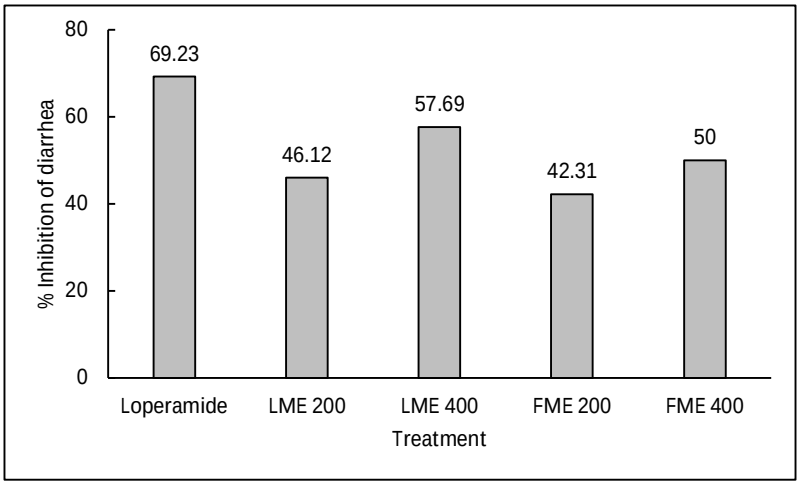

Fig. 3. Anti-diarrheal activity of methanol extract of leaves and fruits of *P. persicaria.*

Table 4. Hypoglycemic activity of methanolic extracts of leaves and flowers of *P. persiceria*.

Sample	Mean plasma level of glucose (mmol/l)			
	0 min	1 h	2 h	3 h
Control	6.4	6.7	7.3	6.5
Glibenclamide	6.2	3.5	2.8	2.5
LME 200	5.2	4.6	3.4	3.0
LME 400	5.7	4.8	4.4	4.4
FME 200	6.2	4.9	4.4	4.0
FME 400	5.6	4.9	4.0	3.8

Table 5. CNS anti-depressant effect of methanol extracts of leaves and flowers of *P. persicaria*.

Group	Onset of sleep (min.)	Duration of sleep (min.)
Control	17.33	86.0
Standard	16.33	172.0
LME 200	63.67	116.33
LME 400	55.67	131.33
FME 200	68.0	130.33
FME 400	63.67	177

A variety of neuropharmacological experimental models are available to investigate the CNS activity, including the hole cross test, open field test, phenobarbitone-induced sleeping time test, and muscle relaxant activity test. In addition to these broad behavioral characteristics, experimental animals can additionally be evaluated for righting reflex, pinna reflex, grip strength,

touch sensitivity, pain reaction, and sound response. The current work used phenobarbitone sodium-induced sleeping time test to examine the CNS anti-depressant efficacy of methanol extract of *P. persicaria* leaves and flowers in albino mice. When leaf extract was administered at doses of 200- and 400 mg/kg b.w., the time of sleep onset was 63.67 min and 55.67 min in the experimental group, respectively (Table 5). After taking 200 and 400 mg/kg b.w., accordingly, the subjects slept for around 116.33- and 131.33 min total. While floral extract was administered at 200- and 400 mg/kg b.w., the onset and total amount of time spent sleeping were 68 and 63.67 min, correspondingly (Table 5). The above results indicated that both extracts decreased the locomotor activity as revealed by prolonged sleeping time mice. Sedation effect may be due to interaction with benzodiazepines-like compounds. The leaf extract and flower extract might have acted by potentiating GABAergic inhibition in the CNS.

According to the study, *P. persicaria* contains bioactive substances such phenol, flavonoids, and alkaloids that possess antidiarrheal, cytotoxic, analgesic, and antioxidant properties. To identify *P. persicaria's* original mechanisms and isolate possible phytoconstituents, further comprehensive investigation is recommended.

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