Original Research Article

In-Vitro and In-Vivo Pharmacological Evaluation of *Persicaria lapathifolia* Available in Bangladesh

Purpose: The purpose of this research was to examine the effects of *Persicaria lapathifolia* (Pale smartweed) leaf and bark extracts prepared with methanol on in vitro activities like antioxidant, antimicrobial, anti-arthritis, anti-inflammatory, and cytotoxic, as well as on some in vivo activities like analgesic and neuropharmacological properties in an animal model.

Methodology:

The primary method of assessment for the methanolic extract of *Persicaria lapathifolia* leaf (MEPLL) and methanolic extract of *Persicaria lapathifolia* bark (MEPLB) were investigated for phytochemical screening. It was determined to look into the plant's potential for in vitro activities, such as phytochemical screening, antioxidant, anti-arthritis, anti-bacterial, and cytotoxic, due to the pharmaceutical interest in its component parts. Its described effects on its in vivo analgesic and neuropharmacological activities in animal models are significant compared to the standard. This research was conducted between July 2022 and November 2022 in the Stamford University Bangladesh, Dhaka, Laboratory of Physiology and Pharmacology, Department of Pharmacy, and Laboratory of Microbiology. Antioxidant, anti-arthritic, anti-inflammatory, antibacterial, cytotoxic, analgesic, and neuropharmacological activities of MEPLL and MEPLB were investigated. Phytochemical screening was performed utilizing a battery of test reagents. Antioxidant, total phenolic content, anti-arthritic, anti-inflammatory, antibacterial, and cytotoxic activities were measured at various doses using the DPPH Free Radical Scavenging Assay, the Folin-Ciocalteu technique, the Inhibition of Protein Denaturation Assay, Egg Albumin Protein Denaturation Assay, the Disk Diffusion Method, and the Brine Shrimp Lethality Bioassay. The analgesic effectiveness was evaluated by using acetic acid to elicit writhing in *Swiss albino mice* and then testing the animals on a hot plate. Locomotor activity was measured by open field and hole cross tests as part of the neuropharmacological investigation.

Results:

Research performed on MEPLL and MEPLB's phytochemical screening yielded results that revealed the presence of a variety of different phytochemical components. In comparison to MEPLL's IC_{50} value of 1.49 µg/mL, MEPLB's value of 30.45 µg/mL indicates its superior antioxidant activity. Both extracts demonstrated significant anti-arthritic and anti-inflammatory effectiveness in comparison to the standard drug. When compared to MEPLB, MEPLL exhibited much more antibacterial activity. In a lethality test using brine shrimp, MEPLL and MEPLB both had much higher LC_{50} values than the standard vincristine sulfate. Their respective values were 1.42 and 0.66 µg/mL. In contrast, MEPLL inhibited peripheral nociception in the acetic acid-induced writhing nociceptive paradigm with percent inhibitions of 60.72 and 67.56, respectively, while MEPLB inhibited 48.22 and 67.28, respectively, at the aforementioned doses. In addition, the hot plate test revealed a significant analgesic effect. The extract was also shown to have a more negative impact on motor coordination in the Open Field and Hole Cross Tests than the standard Diazepam.

Conclusion:

This research demonstrates a relationship between the existence of anti-oxidative, anti-arthritis, and anti-bacterial activities, as well as a strong cytotoxic, analgesic, and neuropharmacological activity, which substantiates all previously made claims against *Persicaria lapathifolia*.

Keywords: *Persicaria lapathifolia*, antioxidant, antiarthritic, antitumor, analgesic.

1.0 Introduction

Traditional usage of medicinal plants that have been recorded throughout time continue to be an essential source for the treatment of a broad variety of conditions. The majority of Ethiopia's population seems to continue to rely on traditional medicine, according to the available evidence [1]. The existence of active chemicals in medicinal herbs is crucial to their therapeutic value. Essential oils, saponins, alkaloids, terpenoids, phenolic compounds, flavonoids, and tannins are some examples of biomolecules which are the reason behind a plant's medicinal activity. There are over 150 herbaceous species of *Persicaria*, which are found all over the world. These plants are generally known as the smartweeds or knotweeds. The vast majority of species may be found in temperate climates; however, some can be found in tropical and subtropical regions at sea level and at a variety of various elevations [2]. In some regions of Ethiopia, traditional medical practitioners often consult with the plant species known as *Persicaria lapathifolia*, which belongs to the family Polygonaceae and is used to make medicine. In spite of the widespread use of this plant in traditional medicine for the treatment of a broad variety of conditions, the phytochemical information associated with the seeds of this plant and the microbiological activity associated with it have not been investigated [3] [4].

The biennial plant *Persicaria lapathifolia* has scarlet stems and alternating leaves and may reach a height of 80 cm [5]. In addition to being used to treat burns, dysentery and fevers, *Persicaria lapathifolia* possesses antimicrobial, anti-inflammatory, antiviral, astringent, anti-stomach complaint, antiseptic and hepatoprotective applications [6]. Different extracts and isolated metabolites from this plant have been shown to have antibacterial, antioxidant activity in a number of investigations [7].

On the basis of the evidence presented, the current research will concentrate on the effects that the leaves and bark of *Persicaria lapathifolia* exert in vitro as an antioxidant, antimicrobial, and cytotoxic assay, as well as in vivo as an analgesic and neuropharmacological test in several different models using mice.

2.0 MATERIALS and METHODS

2.1 Plant materials:

The sample plant *Persicaria lapathifolia* was taken in July 2022 from West-Delpara, Kutubpur, Narayanganj, Dhaka. The professionals at the Bangladesh National Herbarium in Mirpur, Dhaka, were able to correctly identify the plant (accession number: DACB87208). The leaves and bark of the plants were dried in the shade and then processed into a powder.

2.2 Drugs and Chemicals

Sigma Chemical Co., USA provided methanol, NaOH, dilute HCl acid, concentrated H_2SO_4 and acetic acid. Gonoshasthaya Pharmaceuticals Ltd. supplied the morphine sulphate (Dhaka, Bangladesh). Our Vin-Cristine Sulphate came from Celon Laboratories Pvt. Ltd. India. It was through Orion Infusion Ltd. that we acquired the sterile saline solution. Diazepam and diclofenac sodium injections were produced by Square Pharmaceuticals Ltd. The DMSO came from the German business Merck. Bovine Serum Albumin was purchased from Polysciences, Inc. India.

2.3 Preparation of extraction

By removing the soil, fresh Leaves and bark of the *Persicaria lapathifolia* plant were harvested. Then, to thoroughly remove the dust, the leaves and barks were rinsed in water that was at normal

temperature. Leaves and barks of *P. lapathifolia* were cleaned, then air dried for 12 to 15 days in the shade. Leaves and barks were chopped into little pieces and blended by a machine once they had sufficiently dried. 900 mL of methanol were used to soak 985g of powdered *P. lapathifolia* for three days. Throughout this time, the maceration process was occasionally aided by stirring.

The extract was filtered using filter paper and then cotton after three days. 157.67g of leaf extract and 141.9g bark extract were produced using a rotary evaporator (BC-R 201 Shanghai Biochemical Equipment Co. Ltd.) to remove the solvent. The unprocessed extract was kept in a beaker and kept cold and out of the sun.

2.4 Phytochemical screening

The presence of certain chemical components in the freshly prepared MEPLL and MEPLB extract was evaluated qualitatively. The extract was subjected to qualitative phytochemical assays, as indicated above, to identify tannins, flavonoids, steroids, terpenoids, gum and carbohydrates, saponins, reducing sugar and alkaloids [8] [9].

2.5 Analysis of Antioxidant Potential

2.5.1 DPPH free radical scavenging assay

The DPPH radical scavenging capacity was determined using the procedure as it was described before, with a few minor adjustments [10]. In this investigation, a variety of concentrations of MEPLL and MEPLB extracts, in addition to the standard ascorbic acid (500, 250, 125, 62.5, 31.25, 15.62, and 7.81 µg/mL), were used. These concentrations ranged from low to high. The following is the formula that should be used for calculating the percentage of the free radical that has been inhibited [34,35]. The formula for calculating the percentage of inhibition is as follows:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of blank} - \text{Absorbance of Test sample})}{\text{Absorbance of blank}} \times 100$$

2.5.2 Total phenolic content:

Total phenolic content was calculated using a modified version of the Folin Ciocalteu test [11]. Following a 5-minute incubation at room temperature, 40 µL of the leaf extract was combined with 900 µL of diluted Folin Ciocalteu's reagent. After adding 400 µL of 15% sodium carbonate, the mixture was left to react at room temperature for 45 minutes. The blank solution was then used to determine the UV absorbance at 752 nm. Gallic acid standard solution was used to determine the standard curve, and the result was expressed as mg of gallic acid per µg SD. The mean of three independent experiments was used to determine the outcome.

2.6 In vitro anti-arthritic activity

2.6.1 Protein denaturation assay

The "inhibition of protein denaturation" (REF) assay was used to measure MEPLL and MEPLB extract's anti-arthritic efficacy in vitro, with diclofenac sodium serving as the standard. An aqueous solution of 5% bovine serum albumin and 0.05 mL of the test solution equals 0.5 mL of total solution (MEPLL and MEPLB extracts). The 5% w/v aqueous solution of bovine serum albumin and distilled water make up the remaining 0.05 mL of the 0.5 mL test control solution. The control product (0.5 mL) is made up of 95% distilled water and 5% test solution. Bovine serum albumin (5% w/v aqueous solution) and diclofenac sodium (0.05 mL) make up the 0.5 mL standard solution. Diclofenac sodium (the gold standard) and MEPLL and MEPLB extracts were sampled at 62.5, 125, 250, 500, and 1000 µg/mL, respectively. Using 1 N HCl, the pH of all the solutions was lowered to 6.3. After 20 minutes incubation at 37 degrees Celsius, the temperature was raised to 57°C and held there for 3 minutes. As soon as the solutions had cooled, 2.5 mL of phosphate buffer were added. A UV-Visible spectrophotometer was used to determine the absorbance at a wavelength of 416 nm. Proteins are denatured to their fullest extent under the control condition. Diclofenac sodium was used as a comparison in this study. **Table 4** summarizes the proportion of protein denaturation inhibition at various doses. Formula for determining protein denaturation inhibition percentage:

% Of Inhibition = [100 - (OD of test solution - OD of product control) x100]

In which optical density (OD) is the descriptor

Proteins are denatured to their fullest extent under the control condition. Diclofenac sodium was used as a comparison in this study.

2.7 In vitro anti-inflammatory assay

The final concentrations of the extract were 100, 200, 300, 400, and 500 µg/mL, and the reaction mixture (5 mL) included 0.2 mL of egg albumin (from a fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4), and 2 mL of different concentrations of extract. As a comparison, a control group drank a same amount of double-distilled water. After mixing, the substances were placed in a BOD incubator (Lab line Technologies) for 15 minutes at (37°C) before being heated at 70°C for 5 minutes. Their absorbance was checked at 660 nm (SHIMADZU, UV 1800) after they had cooled down, with the vehicle serving as a blank. As a standard, we measured the absorbance of a solution containing 100, 200, 300, 400, and 500 µg/mL of acetylsalicylic acid. The formula for determining the proportion of protein denaturation that was prevented is as follows:

$$\% \text{ Inhibition} = \frac{Abs\ of\ control - Abs\ of\ test\ sample}{Abs\ of\ control} \times 100$$

2.8 Antimicrobial Test

2.8.1 Test Microorganisms

The Department of Microbiology at Stamford University Bangladesh supplied pure cultures of Gram-positive (*Staphylococcus aureus, Bacillus megaterium*) and Gram-negative (*Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa*) bacterial pathogens.

2.8.2 Antibacterial Susceptibility Test

Using the disc diffusion technique [12], the antibacterial activity of MEPLL and MEPLB was assessed. In the experiment, both gram-positive and gram-negative bacterial strains took part. In Table 5 we can see all the bacterial strains that were tested. MEPLL and MEPLB were dissolved in the appropriate volumes of solvents to produce solutions of known concentration (300, 500 and 700 µg/mL). We used a micropipette to saturate dried, sterile filter paper discs (5.0 mm in diameter) with predetermined concentrations of the test chemicals. The test microorganisms were evenly seeded onto nutrient agar medium, and discs containing the test material were then put on the discs. A standard antibiotic disc containing Ciprofloxacin at the same concentration was employed as a positive control, while blank discs (impregnated with solvents) served as a negative control. The plates were placed in a 37°C incubator for 24 hours to promote optimal microbial growth. The antibacterial test materials slowed the spread of germs, and a clear, definable zone of inhibition could be seen all the way around the medium. In order to quantify the antibacterial efficacy of the test agents, we measured the zone of inhibition diameter in millimeters. The procedure was repeated three times, and the average result is were considered.

2.9 Cytotoxicity Test

2.9.1 The Brine Shrimp Lethality Bioassay

The cytotoxic effects of MEPLL and MEPLB were evaluated using the standard bioassay for screening bioactive compounds, the brine shrimp lethality test [13] [14]. Zoological organisms were *Artemia salina*, which was employed as a model in this investigation. To begin, brine shrimp eggs were purchased from a pet store (Dhaka, Bangladesh). After 48 hours of incubation in artificial seawater (3.8% NaCl solution), brine shrimp hatched and grew into larval shrimp (nauplii). This evaluation of brine shrimp nauplii for cytotoxicity employed Meyer's approach. For the preparation of the samples to be tested (MEPLL and

MEPLB), they were dissolved in a dimethyl sulfoxide solution that contained not more than 50 µL per 5 mL. In order to achieve concentrations of 10, 50, 100, 150, 200, 300, and 500 µg/mL, saltwater (3.8% NaCl solution) was added. Then, 5 mL of dimethyl sulfoxide was removed from a bottle and used as a standard. The standard medicine vincristine sulphate served as a positive control in this study. Each test tube contained 10 mature shrimps. After 24 hours, vials were examined under a microscope to see how many nauplii had survived. The LC_{50} was calculated using a logarithmic plot of concentration against death rate.

2.10 Experimental Animals for In-vivo pharmacological investigation

As investigators, we selected young, healthy Swiss albino mice weighing between 20- 26 g. We purchased our mice from Jahangirnagar University at their Saver facility in Dhaka, Bangladesh. Preserving the status quo was essential. Normal atmospheric changes include a temperature of (77° F), a relative humidity of 55 to 65%, and a light/dark cycle of 24 hours every day. Constant conditions are maintained for 8 days after collection. To assist mice, recuperate from the water and food deprivation they experienced during travel and to acclimatize to the laboratory environment, we fed them a diet of adequate food and clean water, as recommended by Jahangirnagar University. After 5 days of recuperation, the mice were ready for the experiment.

2.11 Analgesic Studies

2.11.1 Hot plate test

As described by Lanhers et al. [15] and Ojewole [16], analgesic activity was evaluated using the hot-plate test (Eddy's hot plate). There was a thermostat set to keep the temperature at 51°± 1°C. Mice of both genders were split into four groups filled with 5 individual mice in each group. Each group's mice were put in a beaker (on a hot plate) to measure their reaction to an electrical heat-induced pain stimuli. Behaviors like as licking the paws were recorded as indicators of the animal's reaction to the painful heat. We measured the response time (in seconds) of each mouse by timing how long it took them to lick their paws or escape the beaker. The response time was measured once before any kind of therapy was given. Initial response time before treatment for each mouse group was calculated as the mean of this determination. Following this, each test mouse was orally administered either distilled water (DW), Diclofenac sodium (10 mg/kg BW), or MEPLL and MEPLB at dosages of 200 and 400 mg/kg BW, respectively. Reaction times were measured five times at one-hour intervals beginning 30 minutes after therapy was given in each group of mice. The formula for the analgesic effectiveness of treatment was as follows:

Percent Analgesic Score = $\frac{Ta-Tb}{Ta}$ X100.

Time (in seconds) to react (before medication administration): Tb; Time (in seconds) to react (after drug administration): Ta.

2.11.2 Acetic acid induced writhing test

The analgesic activity of the sample was measured by utilizing the acetic acid induced writhing technique in mice, similar to the approach developed by M. L. Altun et al. [17] and Owoyele et al. [18], with a few modifications. Acetic acid is injected intraperitoneally into the animals used in the experiment, thereby giving the animals a painful experience. Four groups of five mice each were used to study the animals. Animals in Group I were given distilled water, animals in Group II were given Diclofenac sodium (10 mg/kg), and animals in Groups III and IV were given 200 and 400 mg/kg of MEPLL and MEPLB, respectively, following an overnight fast. Thirty minutes before to intraperitoneal injection of 0.7% v/v acetic acid solution, test samples and vehicle were orally delivered. Each animal was housed in its own clear glass container so that researchers could study them. Five minutes after receiving the acetic acid solution intraperitoneally, we started keeping track of how many times each mouse in all groups writhed in

a cage for the next ten minutes. Not all animals produced full writhes; others would start but not finish. It was determined that this kind of writhing was only half complete. Two partial writhes were thus counted as a single complete one. Each group's writhing count was compared to a control group that received a placebo of Diclofenac sodium (positive control). The proportion of writhing restraint was determined by using the following formula:

% Of writhing = $\frac{VC-VT}{VC}$ X 100

VT = number of writhing motions in extract-treated mice. VC = number of writhing motions in the control group of mice.

2.12 Neuropharmacological Studies

2.12.1 Hole cross test

Using the method from Archi et al. [19], a cage with the dimensions 0.30 m x 0.20 m x 0.14 m in their research. A wall was installed along the middle of the space. At a height of 0.075 m, directly in the center of the framework, a hole with a diameter of 0.03 m was cut. The animals used in the experiments were housed in one section of the cage and given either a placebo, a gold standard, or a test sample. At 30, 60, 90, and 120 min after the administration of the control, standard, and experimental extracts, the number of mice passes through the opening from one chamber to the other was counted for 5 min. The subjects in Group I received a placebo (distilled water) while those in Group II received the standard (diazepam). Both MEPLL and MEPLB was given orally to groups III and IV at 200 and 400 milligrams per kilogram of body weight, respectively.

2.12.2 Open Field Method

All of the procedures used in the experiment were taken straight from Habib et al.[20]. Open field one square meter in size was tiled into many squares. A wall of 40 cm in height surrounded the whole setup. After receiving an oral test drug treatment of both MEPLL and MEPLB and intraperitoneal dose of Diazepam, the number of squares visited by the mice was tallied for 5 minutes at 30, 60, 90, and 120 minutes.

2.13 Statistical analysis

All bioassay readings were conducted in triplicate, and the tabular data is provided as the mean standard deviation. Statistical analyses were conducted using Excel.

3.0 RESULTS and DISCUSSIONS

3.1 Phytochemical Screening

The methanolic extracts of the leaf and bark of *Persicaria lapathifolia* were studied for chemical group test. This screening test revealed that Both MEPLL and MEPLB contains reducing sugar, saponins, alkaloids, flavonoids, glycosides, carbohydrates and absence of steroids.

Table 1. Results of Different Chemical Group Test of the leaf and bark of *Persicaria Lapathifolia*

Phytochemical constituent	MEPLL	MEPLB
Flavonoid	+	+
Tannin	+	+
Alkaloid	+	+
Steroid	-	-
Saponin	+	+
Carbohydrate	+	+

Glycoside	+	+
Reducing Sugar	+	+
Gum	+	+

Here, (+) indicates the presence of the phytochemical and (-) indicates the absence of the phytochemical

Tannins, alkaloids, flavonoids, gum and glycoside, reducing sugar, and carbohydrates have been shown to be present in MEPLL and MEPLB, according to the phytochemical screening of both of these substances. The existence of these compounds indicates that there is a potential for the methanolic extracts of the leaf and bark of the *Persicaria lapathifolia* plant to exhibit some type of biological activity.

Studies have shown that flavonoid molecules have a wide range of pharmacological properties. Analgesic and antibacterial properties have been attributed to some flavonoids[21][22]. An explanation for this effect is because it blocks arachidonic acid metabolism by inhibiting phospholipase A2 [23].The metabolic route of arachidonic acid may be blocked by a variety of alkaloids, which may also be effective in reducing inflammation.

3.2.1 DPPH Free Radical Scavenging Assay

It was also determined how much DPPH was inhibited. The results demonstrated a concentration-dependent increase in DPPH radical scavenging activity at 3–30 g/mL with an 8–74% inhibition of DPPH (**Table 2**). Ascorbic acid (used as a reference) was linked to a 50% inhibition of the DPPH radical at 21.86 µg/ml concentration, while 1.49 µg/mL and 30.45 µg/mL of MEPLL and MEPLB was linked to a 50% inhibition.

Table 2. In vitro free radical scavenging effect of MEPLL and MEPLB

Samples	Concentrations	% of Inhibition	IC_{50} in DPPH radical scavenging analysis (µg/mL)
Ascorbic Acid	7.81	55.72	21.86
	15.625	59.67	
	31.25	70.57	
	62.5	73.70	
	125	81.60	
	250	88.55	
	500	93.86	
MEPLL	7.81	48.20	1.49
	15.625	52.51	
	31.25	62.58	
	62.5	65.46	
	125	74.82	
	250	82.73	
	500	92.80	
MEPLB	7.81	45.16	30.45
	15.625	64.51	
	31.25	80.64	
	62.5	83.87	
	125	88.70	
	250	91.93	
	500	93.54	

Antioxidants and free radicals have both beneficial and detrimental effects on health. They might be made locally, by regular cellular metabolism, or externally, through exposure to environmental hazards, tobacco, radiation, or certain drugs. Oxidative stress occurs when an excessive number of free radicals overwhelms a system and cannot be neutralized. Many diseases, including autoimmune disorders, cancer, senescence, cardiovascular disease, and neurodegenerative disease, may have their origins in oxidative stress. However, the body has a number of in situ and ex situ systems to combat oxidative stress from its environment [24]. The capacity of antioxidants like phenolic compounds and flavonoids to scavenge oxygen radicals is measured by this property. For their antioxidant action, phenolic compounds rely largely on their redox characteristics, which allow them to operate as reducing agents, hydrogen donors, and singlet oxygen quenchers. By inhibiting the free radical DPPH, the DPPH test demonstrated that the plant extract had substantial antioxidant activity (1.49 and 30.45 mol Vitamin C/µg). The data was presented as the amount of vitamin C per extract in micromoles.

3.2.2 Total phenolic contents (TPC)

Total phenolic content of MEPLL and MEPLB are shown below in the **Table 3**.

Table 3. Determination of TPC of MEPLL

Samples	Total Phenolic content
Gallic Acid	49.30
MEPLL	83.20
MEPLB	82.09

This investigation used the Folin method to quantify the total phenolic content of MEPLL and MEPLB in terms of mg gallic acid per gram of crude extract by calibrating against a series of known gallic acid values (**Table 3**). In ethanol extraction studies, the TPC of MEPLL and MEPLB plant material was determined to be 83.20 and 82.09 mg/µg. These findings demonstrate the high concentration of polyphenolic chemicals in the plant's ethanolic extract. Phenolic compounds, which are thought to directly contribute to antioxidative activity due to their reputation as potent chain breaking antioxidants and role as free radical terminators, are possible candidates. The presence of hydroxyl groups in these molecules gives plants the capacity to scavenge free radicals, making them an essential component of plant life [25].

3.3 In vitro anti-arthritic activity

Significant percentage reduction in protein denature was seen across a range of doses of MEPLL, MEPLB, and diclofenac sodium, all evaluated for antiarthritic efficacy (**Table 4**).

Table 4. Percentage inhibition in protein denaturation of MEPLL and MEPLB.

Samples	Concentrations	% of inhibition
Diclofenac Sodium	62.5	84.64
	125	87.38
	250	89.18
	500	93.69
	1000	94.59
MEPLL	62.5	72.07
	125	81.98
	250	82.88
	500	90.98
	1000	91.89
MEPLB	62.5	86.48

	125	92.79
	250	94.59
	500	94.59
	1000	95.49

According to previous research, protein denaturation is the primary cause of inflammatory and arthritic illnesses, which then leads to the production of auto-antigens and, ultimately, rheumatic disorders [26]. Hydrogen, electrostatic, disulfide, and hydrophobic bond variations may be involved in the denaturation [27]. Drugs used to treat arthritis inhibit protein denaturation caused by heat in a dose-dependent manner [28]. As a result, chemicals that prevent protein denaturation would be of use in the creation of anti-arthritis medications. Both MEPLL and MEPLB significantly suppressed protein denaturation (BSA) and had more dramatic effects than standard medication in the present study. The results suggest that *Persicaria lapathifolia* may inhibit the generation of auto-antigens, which in turn decreases protein denaturation and helps alleviate arthritis.

3.4 Anti-inflammatory activity

In this test it has been observed that both MEPLL and MEPLB have more significant effect compared to the standard acetyl salicylic acid which has been demonstrated in **Table 5.**

Table 5. Percentage inhibition in egg albumin denaturation of MEPLL and MEPLB.

Samples	Concentrations	% of inhibition
Acetyl salicylic acid	62.5	20
	125	33.33
	250	66.67
	500	77.78
	1000	88.88
MEPLL	62.5	87.61
	125	89.54
	250	89.87
	500	90.51
	1000	93.03
MEPLB	62.5	89.54
	125	89.87
	250	90.00
	500	90.45
	1000	90.64

To test *Persicaria lapathifolia* for its anti-inflammatory effects, the anti-denaturation of egg albumin technique was used. The egg albumin used in the anti-denaturation test is exposed to a heat treatment that causes the protein to denature, or become unusable. Serum sickness, glomerulo-nephritis, and other autoimmune disorders are linked to the denatured protein's expression of antigens associated with Type III hyper-sensitive response. Proteins that have been heat-denatured may still cause delayed hypersensitivity [29]. Moreover, it has been shown that traditional NSAIDs, such as phenylbutazone and indomethacin, do not just decrease endogenous prostaglandins production by inhibiting COX enzyme, but also by preventing denaturation of proteins [23]. As this is the case, the anti-denaturation assay provides a practical way to test for anti-inflammatory properties. The current study's findings suggest that the extract has potent anti-inflammatory properties. The efficacy of *Persicaria lapathifolia* in controlling auto antigen synthesis and, by extension, inhibiting denaturation of proteins, was compared to that of the gold

standard medication Aspirin. Preliminary phytochemical screening identified phenolic chemicals and tannins, which are examples of secondary metabolites, and which may be responsible for this action.

3.4 Antibacterial activity

The antibacterial activity of various doses of plant extract was studied against gram positive and gram-negative bacteria by measuring the zone of growth inhibition in millimeters (mm). According to the findings, a rise in the concentration of plant extract resulted in an expansion of the zone of inhibition (Table 6).

Table 6. Inhibition zone of *Persicaria lapathifolia* methanolic extracts of leaf against microorganism

Diameter of Zone of Inhibition (mm)				
Test organisms	MEPLL (300 µg/disc)	MEPLL (500 µg/disc)	MEPLL (700 µg/disc)	Ciprofloxacin
Gram Positive Bacteria				
Bacillus cereus	09	10	11	25
Staphylococcus aureus	08	09	10	26
Gram Negative Bacteria				
Escherichia coli	07	08	09	25
Pseudomonas aeruginosa	08	09	10	27
Klebsiella pneumonia	07	07	08	24

Table 7. Inhibition zone of *Persicaria lapathifolia* methanolic extracts of bark against bacteria

Diameter of Zone of Inhibition (mm)				
Test organisms	MEPLB (300 µg/disc)	MEPLB (500 µg/disc)	MEPLB (700 µg/disc)	Ciprofloxacin
Gram Positive Bacteria				
Bacillus cereus	10	11	14	25
Staphylococcus aureus	08	10	11	26
Gram Negative Bacteria				
Escherichia coli	09	10	11	25
Pseudomonas aeruginosa	07	10	11	27
Klebsiella pneumonia	09	11	12	24

Using disk-diffusion methods, the antibacterial activity of *Persicaria lapathifolia* plant's leaf and bark extract was tested against five distinct bacteria (three gram-negative and two gram-positive). Leaf and bark of the plant were extracted using methanol at concentrations of 300, 500, and 700 µg/disc, respectively (**Tables 6 and 7**). A different zone of inhibition was observed for gram-negative and gram-positive bacteria (varying from 7 to 14 mm). The bark had the largest inhibitory zone (14 mm) at a dosage of 300 µg/disc. For the reason that certain of the bioactive components of plant extracts, such as secondary metabolites, may have antibacterial properties. Most effective in inhibiting the growth of *Staphylococcus aureus* and *Bacillus cereus*, with zones of inhibition of 10–14 mm, and least effective at inhibiting *E. coli* and *Klebsiella pneumoniae*, measuring 7–11 mm. It's possible that these bacteria have become resistant to antibiotics due to the low amount of ciprofloxacin it takes to eliminate them.

3.5 Cytotoxic Activity

The toxicity of *Persicaria lapathifolia* methanolic leaf and bark extract to brine shrimp was tested using the method described by Khan et al.[30] , and the results are summarized in Table 8.

Table 8. Result of Brine Shrimp Bio-assay and LC_{50} value of MEPLL and MEPLB

Sample name	Concentrations	Mortality %	LC_{50} value
Vincristine Sulphate	7.81	40	20.57
	15.625	40	
	31.25	50	
	62.5	60	
	125	100	
	250	100	
	500	100	
MEPLL	7.81	40	1.42
	15.625	60	
	31.25	60	
	62.5	70	
	125	100	
	250	100	
	500	100	
MEPLB	7.81	10	0.66
	15.625	30	
	31.25	40	
	62.5	60	
	125	80	
	250	100	
	500	100	

The LC_{50} values for MEB, MEL, and respectively were 20.57 µg/mL, 1.418 µg/mL and 0.664 µg/mL. There was a clear death toll among the brine shrimp nauplii in all of the test samples.

However, various dosage levels of the test samples were shown to have variable degrees of mortality to *Artemia salina*. The LC_{50} values for the extract ranged from 0.78125 µg/mL (significant) to 500 g/mL (very significant), demonstrating a clear correlation between concentration and LD50. At a concentration of 500 µg/mL, the death rate was highest and at a concentration of 0.78125 µg/mL, it was lowest. Simply put, as the concentration of the test samples rose, so did the rate of fatality.

In comparison to the go standard vincristine sulphate (20.57 µg/mL), the methanolic extract of the leaves and bark of *Persicaria lapathifolia* demonstrated considerable cytotoxic effect against brine shrimp nauplii at concentrations of 1.4187 and 0.664 µg/mL, respectively. If MEPLB and MEPLL show comparable cytotoxicity to the standard vincristine sulphate, then we may use this information to steer our search for more effective antitumor and pesticide compounds.

3.6 Analgesic Investigation

3.6.1 Hot plate test

In this test the MEPLB and MEPLL displayed a potent to moderate and dose dependent analgesic activity compared to the standard Morphine Sulphate. The analgesic activity of extract found to be little more than half of that of the standard. The mean value of the time tolerance of those extract and standard are given below the **Table 9**.

Table 9. Primary Data Table for Hot Plate Test for Plant Extract of MEPLB and MEPLL

Reaction time at different time intervals (in sec)

Group	Average wt. of mice (g)	30 min	60 min	90 min	120 min
Control	20 to 26	6.4	7.6	6.0	5.4
Morphine (5mg/kg)		8.6	9.4	11.0	6.6
MEPLL (200mg/kg)		4.8	7.0	10.0	12.0
MEPLL (400mg/kg)		6.4	8.6	10.6	11.4
MEPLB (200mg/kg)		9.2	9.4	11.6	12.0
MEPLB (400mg/kg)		7.8	8.4	8.5	14.8

The hot plate test is a paradigm that is used to identify compounds that exhibit a centrally acting analgesic effects. Several animal species have shown that the hot plate method is selective for opioid related compounds [31] [32]. There is speculation that these molecules are responsible for the narcotic analgesic, anti-inflammatory, and anti-diabetic effects. The demonstration of a dose-dependent increase in latency time against thermal pain by MEPLL and MEPLB provided evidence that the same mechanism might be responsible for mediating the analgesic activity action of both MEPLL and MEPLB.

3.6.2 Acetic acid-induced writhing test

In acetic acid-induced writhing test, the present inhibition the methanolic extracts of leaf and bark of *Pescaria lapathifolia* were found to be respectively 60.72%, 67.56%, 48.22%, 67.28, where the standard drug Diclofenac-Na found to be 80.36%. Compared to standards, the result displayed significant analgesic activity which is dose dependent.

Table 10. Analgesic Activity of MEPLL and MEPLB on Mice by Writhing Test

Administered Substance	Dose	% Writhing	% Of Inhibition
Control	10mL/kg	100	0.00
Diclofenac sodium	10mg/kg	20.40	79.61
MEPLL	200mg/kg	39.28	60.72
MEPLL	400mg/kg	32.14	67.56
MEPLB	200mg/kg	51.78	48.22
MEPLB	400mg/kg	32.72	67.28

The current research revealed that *Pescaria lapathifolia* extract had analgesic action [33] in the two analgesic models, indicating the existence of both centrally and peripherally mediated pathways. *Pescaria lapathifolia* extract effectively and dose-dependently decreased abdominal writhing in an acetic acid-induced abdominal constriction test. The test is effective for evaluating moderate analgesic non-steroidal anti-inflammatory medications since acetic acid is thought to operate indirectly by causing the release of prostaglandins and lipo-oxygenase products into the peritoneum, which activate the nociceptive neurons responsive to the NSAIDs. The results of the acetic acid-induced writhing strongly suggest that the mechanism of this action is linked in part to inhibition of lipo-oxygenase and/or cyclooxygenase in the

peripheral tissues, thereby decreasing prostaglandin synthesis and interfering with the mechanism of transduction in primary afferent nociceptors.

3.7 Neuropharmacological Activity

3.7.1 Open field test

Open field test showed that the depressing action of the extracts was evident from the second observation period in the test animals at the both doses of 200 mg/kg and 400 mg/kg body weight.

Table 11. The primary data table for the open field test for the both MEPLB and MEPLL

Number of movements (Mean value)					
Group	Average wt. of mice (g)	0 min	30 min	60 min	90 min
Control	20 to 26	89.6	39	32.4	22.8
Diazepam		87.2	62	36.2	18.8
MEPLL (200mg/kg)		67.6	55.6	49.4	48.2
MEPLL (400mg/kg)		56.6	41.4	33.2	16.8
MEPLB (200mg/kg)		72.2	43.6	29.4	25.4
MEPLB (400mg/kg)		74.6	36.0	33.2	16.4

3.7.2 Hole cross test

Spontaneous movement of the animals through the hole from one chamber to the other was counted for 5 minutes in this test. The observations were made on 30, 60, 90 and 120 minutes after intraperitoneally injection of the leaves extract of the *Persicaria lapathifolia*. There were no effects of the test animals at 0 min. After 30 min observed that the mice began to sleep and therefore very little movement was observed. Even after 90 min of administration of the extract they were still sleeping. The extracts significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident for the both doses of 200mg/kg and 400 mg/ kg body weight at the 2nd observation (60 min) and continued up to 3rd and 4th observation (90 and 120 min) period. Moreover, the validation of anxiety was carried out by measuring external signs, through hole-cross tests

Table 12. The primary data table for the Hole Cross Test for the MEPLB and MEPLL

Number of movements (Mean value)					
Group	Average wt. of mice (g)	30 min	60 min	90 min	120 min
Control		23.8	24.4	23.8	19.4

Diazepam	20 to26	9.67	6.67	3.67	2.33
MEPLL (200mg/kg)		14.4	4.8	1.2	0.6
MEPLL (400mg/kg)		9.8	4.0	0.6	0.00
MEPLB (200mg/kg)		9.8	7.8	7.6	3.2
MEPLB (400mg/kg)		10.2	7.8	6.8	1.2

The impact of a medicine on the animal's locomotor activity is a crucial part of evaluating the drug's action on the central nervous system. Activity is a measure of CNS excitability, therefore a reduction in it may be indicative of CNS depression and, by extension, sedation. In order to measure sedative action, this research used open field and hole cross tests. The data demonstrated a dose-dependent reduction in locomotor activity after administration of the extract. At the second (30-minute) observation, the impact on locomotor activity was already noticeable, and it persisted until the fourth observation (90 min). In addition, the hole-cross test was used to objectively measure the sedation's effects on the patient's outward symptoms. Experiments with open-field dosages of 200 and 400 mg/kg body weight revealed that the extract's depressant effect was also visible in the test animals beginning in the second period of observation (Table 5). Our preliminary phytochemical analysis hints to the presence of alkaloids, glycosides, flavonoids, and tannins, any one of which may be responsible for the plant's neuropharmacological action. A decrease in the number of holes crossed by mice from the second to the fourth observation in the hole cross test is indicative of the calming effect of EEGP. The GABA-benzodiazepine receptor may be involved in the process.

4.0 CONCLUSION

It is possible to reach the conclusion that the methanolic extract of *Persicaria lapathifolia*'s leaf and bark exhibits antioxidant, anti-arthritis, antibacterial, cytotoxic, analgesic, and neuropharmacological action. The results of the phytochemical tests revealed that the methanolic extract of the leaf and bark included reducing sugar, as well as saponins, tannins, flavonoids, alkaloids, and glycosides. The bark and leaf extract both exhibited a trace amount of antibacterial activity. The research results of the bioassay on the lethality of brine shrimp revealed that there was high cytotoxicity. Both the writhing test and the hot plate test demonstrate that this plant have some analgesic effect. In addition to this, it was determined that a larger dosage is more effective than a smaller dose. The plant has a considerable influence on the central nervous system. The CNS impact of this plant has been studied and reported in a few publications.

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6.0 COMPETING INTERESTS

The authors have confirmed that they have no conflicts of interest.

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8.0 ETHICAL APPROVAL

All experiments were reviewed by a relevant ethics committee and executed in conformity with all ethical guidelines, as the authors thus attest.

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