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Biological investigation on *Rumex pulcher* available in Bangladesh

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Abstract
The objective of this study was to investigate specific *in vitro* and *in vivo* properties of plant extracts derived from *Rumex pulcher* using the methanol extraction method. The primary method of assessment for the methanolic extract of *Rumex pulcher* (MERP) was established to examine the plant's *in vitro* capacity for various activities, including antioxidant, antimicrobial, cytotoxic, antinociceptive, and neuropharmacological activities. The reason for this phenomenon can be attributed to the pharmaceutical industry's keen interest in the various components of the plant. The DPPH Free Radical Scavenging Assay was employed to assess the antioxidant activity at various dosages. The disc diffusion method was utilized to assess the antimicrobial activity of the plant under investigation. Additionally, the Brine Shrimp Lethality assay was employed to determine the cytotoxicity of the plant. To perform *in vivo* antinociceptive testing, the hot plate method and acetic-acid-induced writhing tests were utilized. Involvement of opioid receptor was used for the confirmation of antinociception activity. The research conducted a neuropharmacological experiment employing the hole cross and open field methods. Acute toxicity was measured using Cinnamon oil induced method. The research findings indicate that MERP exhibits antioxidant activity with an IC_{50} value of 2.72 µg/mL. Additionally, the antimicrobial test revealed a dose-dependent zone of inhibition ranging from 08-18 µg/disc. The cytotoxic trial demonstrated a highly potent LC_{50} value of 1.28. The findings from the hot plate test and the acetic acid-induced writhing test demonstrated a significant antinociceptive effect, with a percentage of inhibition of 53.62% and 57.33% observed at doses of 200 and 400 mg/kg, respectively. Opioid receptor induced model confirmed the effectiveness of antinociceptive activity. The neuropharmacological investigation has demonstrated promising effectiveness. Acute toxicity test demonstrated mortality of the mice was increased after 2000mg/kg. In conclusion, the phytochemical found in this plant demonstrates robust pharmacological characteristics, rendering it a promising contender for drug exploration across diverse domains.

Keywords: Antioxidant, antimicrobial, cytotoxic antinociceptive, neuropharmacological

1. Introduction
The pharmaceutical industry and drug development have relied heavily on medicinal plants for ages. Plants' natural products have provided a wealth of potential drug-like molecules. It is estimated that more than half of all pharmaceuticals in use today originate from natural sources which include plants (Cragg & Newman, 2013) [5]. This represents the importance of medicinal plants in the drug discovery process. Historically, humans have relied on medicinal herbs ever since. Many of the plants used in traditional medicine have been shown to have substantial therapeutic potential via scientific study. Because of technological advancements, researchers have been able to extract the active components in these plants and use them to create new medications.
Aspirin, for instance, is made from the salicylic acid found in the bark of willow trees and is therefore an example of a medication developed from a medicinal plant. As an anti-inflammatory and pain reliever, this substance has been used for centuries, and its discovery ushered in a new era in medicine (Los, n.d.).
Medicinal plants are very essential to the drug discovery process, as well as in the drug production process. The pharmacological characteristics of plant extracts are investigated, and active molecules are extracted and tested for their medicinal potential. Many novel medications have been discovered by this method; for example, quinine (Derived from the Cinchona tree), reserpine (Derived from the Rauwolfia serpentina plant), and vincristine (derived from the Madagascar periwinkle) are all effective treatments for various diseases. Medicinal plant studies have also inspired novel approaches to medication discovery and development. In order to quickly test a large number of substances for their possible therapeutic benefits, high-throughput screening technologies have been created

(Newman & Cragg, 2016) [19]. *Rumex pulcher*, commonly known as fiddle dock, is a herbaceous plant that belongs to the family Polygonaceae. It is native to Europe and western Asia and has been introduced to North America, Australia, and New Zealand (Raycheva *et al*., 2007) [21]. The plant has a stem that may reach a height of 1.2 meters and a basal rosette of oblong to lanceolate leaves that have a unique fiddle shape at the base. The leaves are dark green in color and have wavy borders. They are also coated with tiny hairs. The reddish-brown, hairless stems contain nodes at regular intervals. In long, thin panicles that rise above the leaves, the tiny, greenish-red flowers are grouped. Perennial *Rumex pulcher* plants like wet, well-drained soils and some shade. Common locations for it include wastelands, pastures, meadows, and the sides of highways. Due to its tendency to swiftly spread via its vast root system and its capacity to colonize damaged environments, the plant is sometimes regarded as a weed. It has been used in traditional medicine for a variety of diseases, including skin issues and digestive difficulties, but it is also prized for its decorative features (Li *et al*., 2022) [15]. Investigations have indicated that *Rumex pulcher* contains a variety of bioactive chemicals, such as anthraquinones, flavonoids, and tannins, which have been linked to antioxidant, anti-inflammatory, and anticancer effects. However, there is little information on the medical benefits of this plant. The plant has been used in traditional medicine as a poultice to treat skin issues and as a decoction to treat digestive problems including constipation and diarrhea (Khaliq *et al*., 2023) [13].

In a nutshell *Rumex pulcher* is a herbaceous plant having both decorative and therapeutic uses. Although it is sometimes seen as a weed, its bioactive chemicals have been linked to positive health benefits and it has been utilized in traditional medicine to treat a variety of diseases. To completely comprehend its therapeutic characteristics and prospective uses, further study is required.

The Purpose of this research is to find its potential antioxidant, antimicrobial, cytotoxic antinociceptive, neuropharmacological and toxicological effect of this plant's ethanolic extract.

2. Materials and Methods

2.1 Plant material

In March 2023, a sample plant of *Rumex pulcher* was collected in West-Delpara, Kutubpur, Narayanganj, and Dhaka. The plant was correctly identified by specialists at the Botany Department, University of Dhaka which is situated in Dhaka. The whole plant was dried in the shade for 14 days after accession before being ground into a fine powder for use in the investigation.

2.2 Reagents used in this research

Sigma Chemical Co., USA provided methanol, NaOH, diluted HCl acid, concentrated H_2SO_4 and acetic acid. 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.

2.3 Preparation of plant sample

The extraction process was carried out using the cold maceration technique (Taskin & Bitis, 2016) [27]. After soaking 495 g of plant leaf powder in 900 mL of ethanol for 10 days, the liquid was filtered to get the crude extract. After the solution was filtered, it was air dried for a further nine days. After everything was said and done, there were 37g of crude extracts in total.

2.4 *In vitro* pharmacological investigation

2.4.1 Antioxidant test

2.4.1.1 DPPH radical scavenging activity

The antioxidant activity of the samples was evaluated using the technique provided by Naz and Bano (Naz & Bano, 2013) [18]. This evaluation was conducted on the basis of the samples' ability to scavenge the stable DPPH free radical. The following concentrations of the sample were used: 500, 250, 125, 62.5, 31.25, 15.62, and 7. µg/mL). 1mL of each of these concentrations was added to 3 mL of a 0.1 mmol/L MeOH solution of DPPH. After 30 minutes of observation in the dark, absorbance at 517 nm was measured, and the percentage of inhibitory activity was computed using the formula:

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

2.4.2 Antimicrobial test

2.4.2.1 Test Microorganisms

5 bacteria named *Bacillus cereus, Klebsiella pneumoniae, Vibrio cholerae, Escherichia coli, Streptococcus aureus* were collected from laboratory of microbiology, Bangladesh Council of Scientific and Industrial Research.

2.4.2.2 Antimicrobial susceptibility test

As part of the disc diffusion method, microorganisms were put into petri dishes with Mueller Hinton Agar (MHA) added, and then sterilized discs (6 mm in diameter) were placed into the agar plates [14]. The correct amounts of solvents were used to dissolve MERP to create solutions with known concentrations (300, 500, and 700 g/mL). Then, for a further two hours, the petri dishes were incubated at 4 °C to enable the extracts to permeate the agar. After the petri dishes were incubated at 37 °C for 1 day, the inhibition zones around the discs were measured. The zone of inhibition was measured in cm after 24 hours.

2.4.3 Cytotoxic Test

2.4.3.1 Brine shrimp lethality bioassay

After soaking in sterile sea water for 24 hours, *Artemia salina* cysts were activated in a makeshift aquarium. In each calibrated test tube containing varied dilutions of the MERP, 10 nauplii (48 h old brine shrimp larvae) were sucked through a glass capillary and put. Sterilized sea water was used to get the volume up to 5 mL. Test tubes with sterile saltwater alone, sterile seawater with 1 drop of DMSO, and sterile seawater with 2 and 3 drops of DMSO served as controls in the experiments. These brine shrimp nauplii were then placed in a warm, well-ventilated room with light for 24 hours, during which time they were exposed to several sample solutions. Ten nauplii were placed in a 5 mL solution and observed during periods of inactivity. After incubating the samples for 24 hours, the quantity of dead shrimp was counted by visually inspecting the tubes under a microscope. Genelyn *et al*.'s method was used to test the lethality of papaya leaf extract in brine shrimp (Genelyn G. Madjos, 2019) [8].

2.5 *In vivo* pharmacological investigation

2.5.1 Experimental Animals

2.5.2 Anti-nociceptive test

2.5.2.1 Hotplate test

Response latencies were measured using the hot plate test in accordance with the procedure described in Sulaiman *et al*. The hot plate temperature used in these tests was 55 2 °C

(Ugo Basile, model-7280). Response latency was measured by timing how long it took until an animal put in the Perspex cylinder on the heated surface started licking its rear paws or leaping. 30 minutes before the start of the experiment, the MERP (250 and 500 mg/kg, i.p.) and morphine (5 mg/kg, i.p.) were given. Mice were studied before the drug was given, and then 30, 60, 120, 180, 240, and 300 minutes afterward. Twenty seconds was the limit (Sulaiman *et al*., 2008) [26].

2.5.2.2 Acetic acid induced writhing test

To find out whether the plant extract has analgesic effects, the method described by Shomudro, Shaira *et al*. was used, with minor modifications. 5 mice per group were given either diclofenac sodium (at a dose of 10 mg/kg), plant extract (at doses of 250 and 500 mg/kg), or normal saline solution (at a dose of 10 mL/kg). The placebo saline solution was given to the control group. After the animals had been separated into their respective groups, an injection of 0.7% acetic acid (10 mL/kg) was administered intraperitoneally (i.p.). After an acetic acid injection, the number of writhes (abdominal constrictions) that occurred between 5 and 15 minutes later were tallied. When test animals writhed noticeably less than control group animals, this was taken to indicate an analgesic response (Shomudro *et al*., 2023) [25].

2.5.2.3 Involvement of opioid receptors

To investigate the role of the opioidergic system in the antinociceptive effects induced by MERP, distinct groups of mice (n=5) were administered with a non-selective opioid receptor antagonist, naloxone (5 mg/kg, i.p), 15 minutes prior to the administration of the extract (250 mg/kg; i.p.) and morphine (5 mg/kg). The mice were then subjected to the hot plate and acetic acid tests as previously described (Sulaiman *et al*., 2008) [26].

2.5.3 Neuropharmacological Test

2.5.3.1 Hole cross test

Using Shaira, Shomudro’s methodology, we constructed a cage that was 0.30 m 0.20 m 0.14 m in size. A wall was built along the center of the room. In the middle of the structure at a height of 0.075 m, a hole of 0.03 m in diameter was carved out. The experimental animals were isolated in one area of the cage and given either a placebo, a reference sample, or a test sample. The number of mice passing through the entrance from one chamber to the other was counted for 5 minutes at 30-, 60, 90 and 120minutes following administration of the control, standard, and experimental extracts. Group II got the gold standard (Diazepam), whereas Group I received a placebo (Distilled water). Groups III and IV were administered 250 and 500 mg/kg of body weight of MERP orally, respectively (Shaira *et al*., 2023) [23].

2.5.3.2 Open field test

The experimental methods followed that described in detail by Habib *et al*. The open area was tiled into several one-meter squares. A 40-centimeter-high wall encased the whole structure. For 5 minutes at 30, 60, 90, and 120 minutes after receiving an oral test drug treatment of MERP and an intraperitoneal dosage of Diazepam, the number of squares visited by the mice was recorded (Habib *et al*., 2016) [9].

2.5.4 Acute toxicity test

5 mice in each group were given either 1000 mg/kg, 2000 mg/kg, or 3000 mg/kg of MERP and Cinnamon oil orally, with the vehicle (water) serving as a control. After 24 hours of observation, death rates were obtained for both groups (Franzotti *et al*., 2000) [7].

3. Results & Discussions

3.1 Statical analysis

The results of each and every bioassay were recorded in triplicate, and the data shown in the table represents the average value. Excel was used in the process of carrying out statistical analysis.

3.2 DPPH free radical scavenging assay

Since the DPPH radical is a stable free radical, it may be utilized to predict the action of free radical scavengers. By providing hydrogen or an electron, antioxidants counteract DPPH (Banerjee *et al*., 2005) [1]. Table 1 displays the *Rumex pulcher* methanolic extract's capacity to scavenge DPPH radicals. In comparison to the control butylated hydroxyl toluene (BHT), the extract's DPPH radical-scavenging activity varied from 63 to 86%. The increased amount of phenolic or flavonoid bioactives extracted with a comparatively high polarity-based solvent may be responsible for the enhanced DPPH scavenging activity of the methanolic extract of *R. pulcher* leaves.

Table 1: *In vitro* free radical scavenging effect of MERP

BHT	7.81	55.72	38.31
	15.625	59.67	
	31.25	70.57	
	62.5	73.70	
	125	81.60	
	250	88.55	
	500	93.86	
MERP	7.81	63.33	2.72
	15.625	73.88	
	31.25	76.94	
	62.5	79.72	
	125	80.83	
	250	82.77	
	500	86.38	

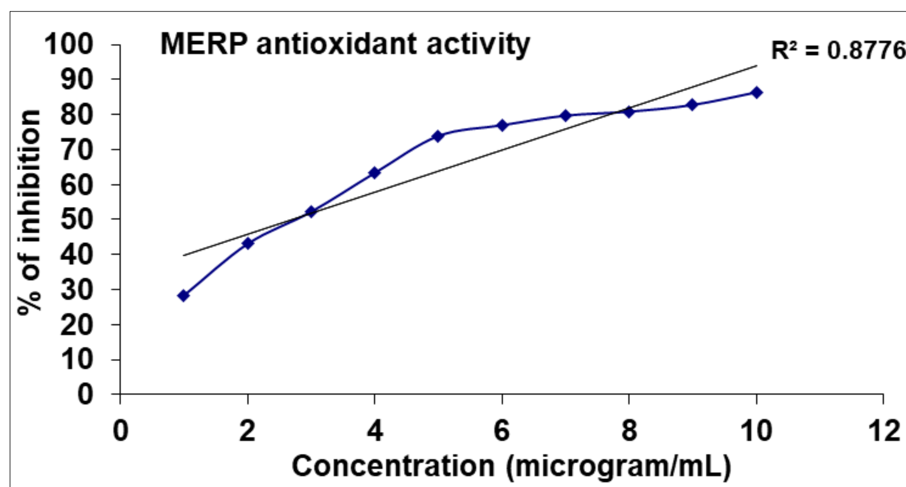

Fig 1: Antioxidant activity of MERP

3.3 Antibacterial test

Table 2 demonstrates that MERP has modest antibacterial activity against all of the examined pathogens. Higher dosage leaves often shown greater action than the others. Extracts had antibacterial activity that ranged from less than 7 mm to no more than 21 mm. MERP was the bacteria that inhibited E. coli the most effectively out of all the microorganisms. The results showed that the microorganisms under study had a significant antibacterial impact in terms of antioxidants. The extracts might be used as natural food preservatives because of their potent antibacterial activities, which are demonstrated by this. A considerable improvement in the antibacterial property for pure compounds was discovered by Belofsky *et al*. (Belofsky *et al*., 2014) [2]. According to this study, extracts that include both antibacterial and antioxidant components may have an enhanced ability to combat pathogenic microbes. Studying the mechanism of this effect and isolating and identifying compounds linked to inhibiting the action of hazardous microbes can be highly helpful.

Table 2: Diameter of zone of inhibition (mm)

Diameter of Zone of Inhibition (mm)				
Test organisms	**MELA (300 µg/disc)**	**MELA (500 µg/disc)**	**MELA (700 µg/disc)**	**Ciprofloxacin**
Gram Positive Bacteria				
Bacillus cereus	14	15	18	25
Staphylococcus aureus	12	14	15	26
Gram Negative Bacteria				
Escherichia coli	13	16	18	25
Vibrio cholera	12	13	14	27
Klebsiella pneumonia	08	09	11	24

3.4 Cytotoxic activity

A. salina was used to investigate whether or not MERP was harmful to a simple zoological organism. In salty aquatic and marine habitats, it is a component of the fauna that consists of invertebrates and is found in aquatic environments. This brine prawn may be used in a laboratory bioassay to test toxicity by estimating the medium lethal concentration (LC50 values). This is done with the use of the medium lethal concentration (MLC) values. The findings on the cytotoxicity are depicted in Figure 2. There was found to be linearity in the dose-effect relationship, which allowed for the calculation of the LC50 value. This was because an increase in mortality was shown to be proportionate to an increase in concentration. In this test, the LC50 value for MERP was 1.28 ng/mL, which was considerably different in comparison to the positive control vincristine sulphate, which had a value of 20.57 ng/mL. This result suggests that the tested extract has a reduced level of toxicity. Therefore, the toxicity of MERP has been evaluated using the brine shrimp fatality bioassay. Scientists from all around the world have used this technique to assess cancer prevention, pest control, and other therapeutic benefits (Matthews, 1995) [14]. It was possible to achieve an LC50 value of less than 10 g/mL thanks to the good connection that existed between the LC50 of the brine shrimp lethality test and the acute oral toxicity experiment in mice. This finding is highly encouraging in terms of the standard cutoff value (Lagarto Parra *et al*., 2001) [14].

Table 3: Result of brine shrimp bio-assay and LC_{50} value of MERP

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	
MERP	0.98	20	1.28
	1.95	20	
	3.91	30	
	7.81	40	
	15.625	40	
	31.25	50	
	62.5	70	
	125	80	
	250	80	
	500	100	

3.5 Antinociception test

3.5.1 Hot plate test

The results of the tests conducted with the hot plate are shown in Table 4. In comparison to the group that served as the control, the latency time (in seconds) was considerably lengthened after oral administration of MERP at dosages of both 250 and 500 mg/kg. A dose-dependent lengthening of the delay period was seen for both study groups. Morphine, when administered at a dose of 10 mg/kg, demonstrated the greatest extension of the latency time. At 90 and 120 minutes, the extracts showed their full potential in terms of their effects.

Table 4: Primary data table for hot plate test for plant extract of MERP

Reaction time at different time intervals (in sec)					
Group	**Average wt. of mice (g)**	**30 min**	**60 min**	**90 min**	**120 min**
Control	22 to 28	6.4	7.6	6.0	5.4
Morphine (5mg/kg)		8.6	9.4	11.0	6.6
MERP (250mg/kg)		9.2	9.4	11.6	12
MERP (500mg/kg)		8.4	8.5	9.2	14.8

The hot plate test is used to assess the centrally-acting analgesics (Imam *et al*., 2012) [12]. The paw-licking or leaping reactions in hot plate are complicated supraspinally coordinated behavior of mice (Chapman *et al*., 1985) [4]. So, a reduction in licking or increase in latency indicates the centrally acting analgesic characteristics of the medication. The findings of the hot plate test demonstrated that *R. Pulcher* extracts provided antinociceptive effect against heat generated discomfort. The impact was visible from the elongation of the latency time to the 5th observation (120 min).

3.5.2 Acetic acid induced writhing test

The conventional medication (Diclofenac sodium) and MERP greatly reduced the writhing response to acetic acid across all dosages, as demonstrated in Table 4. At 500 mg/kg, MEPS showed the greatest percentage inhibition of writhing (57.33%) of any dosage tested. Maximum percentage inhibition (79.61%) was seen with the highest standard medication (Diclofenac sodium).

Table 5: Acetic acid induced writhing test on mice using MERP

Administered Substance	Dose	% Writhing	% Of Inhibition
Control	10mL/kg	100	0.00
Diclofenac sodium	10mg/kg	20.40	79.61
MERP	250mg/kg	46.38	53.62
MERP	500mg/kg	42.67	57.33

The acetic acid-induced writhing test is a standard for measuring the antinociceptive efficacy of both local and systemic analgesics. Endogenous histamine, prostaglandins (PGs), serotonin, bradykinin, cyclooxygenase (COX), lipoxygenase (LOX), and cytokines (TNF-, IL-1, and IL-8) are all released as a result of acetic acid's effect. The major afferent nociceptors in the central nervous system are activated by these systemic inflammatory mediators and results in induction of pain expressing abdominal constriction (Santos *et al*., 2013) [22]. The number of acetic acid-induced abdominal constrictions in mice was dramatically decreased after oral administration of MERP (Table 4). The findings show that MERP's anti-nociception is caused by its suppression of COX, LOX, and other endogenous inflammatory mediators, as well as the signal transduction from primary afferent nociceptors. There is evidence that central and peripheral pathways contribute to phytol's antinociceptive effects (Shajib *et al*., 2015) [24].

3.5.3 Involvement of opioid receptors

A plant species known as *Rumex pulcher* has historically been employed for its analgesic effects in several regions of the globe. It has been discovered that the plant's active ingredients interact with the opioid receptors in the body, which play a role in pain regulation. Numerous studies have been conducted on the role of opioid receptors in the dose-dependent antinociceptive action of *Rumex pulcher*. Numerous research has looked at how various dosages of *Rumex pulcher* extracts affected nociception in animal models. The outcomes have repeatedly shown that *Rumex pultcher's* antinociceptive activity is dose-dependent, with larger dosages causing stronger analgesic effects. This shows that the active components of the plant have a dose-dependent effect on how they interact with the opioid receptors. Additionally, it has been shown that using opioid receptor antagonists, such as naloxone, blocks the antinociceptive effects of *Rumex pulcher* extracts. This provides further evidence that opioid receptors are involved in the analgesic effects of the plant. The endogenous opioid system, which is a key component in pain regulation, includes the opioid receptors. Endogenous opioids, including endorphins and enkephalins, interact with opioid receptors in the body to reduce pain perception. The same receptors are also affected by exogenous opioids like morphine and other opioid medications, which result in analgesia. Mu, delta, and kappa are the three subtypes of opioid receptors. Each subtype is unique pharmacologically and participates in various elements of pain regulation. According to studies, *Rumex pultcher's* antinociceptive effect is mostly mediated by the mu-opioid receptors, with modest contribution from the delta-opioid receptors. (Bunman, n.d.) used the hot plate and writhing tests on mice to examine the antinociceptive properties of *Rumex pulcher*. According to the findings, the plant extract's antinociceptive action was dosage-dependent, with the highest dose (500 mg/kg) having the most analgesic effects. Additionally, the mu-opioid receptor antagonist naloxone but not the delta-opioid receptor antagonist naltrindole or the kappa-opioid receptor antagonist nor-binaltorphimine prevented the extract's antinociceptive effects. These results imply that the mu-opioid receptors play a major role in *Rumex pultcher's* antinociceptive action. The analgesic effects of several *Rumex pulcher* extracts were examined in another research by (Hamidpour *et al*., 2017) [10] utilizing the mouse acetic acid-induced writhing test. All of the extracts provided noticeable analgesic effects, according to the data, with the ethanol extract having the most efficacy. Further evidence that opioid receptors are involved in the plant's analgesic function comes from the fact that naloxone was able to partly negate the analgesic effects of the ethanol extract.

3.6 Neuropharmacological activity

3.6.1 Hole Cross method

According to Table 6, there was a significant decrease in movement during the hole cross experiment for the doses of 250 mg/kg and 500 mg/kg body weight compared to the control group. The maximum decrease in movement was observed at 90 and 120 minutes after drug administration, according to the research findings.

Table 6: The primary data table for the whole cross test for the MERP

Number of movements (Mean value)					
Group	**Average wt. of mice (g)**	**30 min**	**60 min**	**90 min**	**120 min**
Control	23 to 28	23.8	24.4	23.8	19.4
Diazepam		9.67	6.67	3.67	2.33
MERP (250mg/kg)		10.4	10.2	6.2	0.87
MERP (500mg/kg)		9.6	6.2	4.4	0.00

Additionally, considering that the extract suppressed both peripheral and central pain mechanisms, it is plausible that the extract interacted with opioid receptors (Pal *et al*., 2010) [20]. During the evaluation of the neuropharmacological activities of MERP, it was discovered that the plant extract has an impact on the central nervous system. The study shows that there is a depressant activity observed in mice, which is indicated by a decrease in their exploratory behaviour (Dash *et al*., 2015) [6].

3.6.2 Open Field method

The study found that the greatest reduction in movement occurred 90 and 120 minutes following the administration of the drug. In the open field test, it was observed that both doses of the extract (250mg/kg and 500mg/kg) significantly suppressed the number of squares traveled by the mice compared to their initial score. This effect was comparable to the standard drug Diazepam, as shown in Table 7. The maximum suppression was observed at 90- and 120-minutes following drug administration, according to the findings.

Table 8: The primary data table for the open field test for the MERP

Number of movements (Mean value)					
Group	**Average wt. of mice (g)**	**0 min**	**30 min**	**60 min**	**90 min**
Control	22 to 26	89.6	39	32.4	22.8
Diazepam		87.2	62	36.2	18.8
MEPLL (250mg/kg)		68.6	51.6	39.4	28.2
MEPLL (500mg/kg)		52.6	40.2	21.2	6.8

The findings of this study indicate that the *R. pulcher* extract exhibits potent analgesic and CNS depressant properties. These results support the traditional medicinal use of the plant in treating diseases associated with inflammation.

3.7 Acute toxicity test

In the acute toxicity test, administration of the methanolic extract at dosages of 1000, 2000, and 3000 mg/kg as well as cinnamon oil at a dose of 20 mg/kg prolonged the start time of seizure in comparison to the negative control group. The rate of convulsion survivors to total animals tested (mortality protection) was 4/5 in 1000 mg/kg methanolic extract; 3/5 in 2000 mg/kg methanolic extract; and 3/5 in 3000mg/kg methanolic extract, which was less effective than the effectiveness of Cinnamon oil (5/5 in 20 mg/kg).

Table 9: Effect of MERP on mice.

Treatment	Onset time of seizure (s)	Mortality protection after 30min	Mortality protection after 24h
Normal saline	27±2.95	0/5	0/5
Cinnamon Oil (20mg/kg)	343±3.76	5/5	5/5
Methanolic extract (1000mg/kg)	19±2.21	0/5	1/5
Methanolic extract (2000mg/kg)	75±3.04	1/5	2/5
Methanolic extract (3000mg/kg)	115±2.82	2/5	2/5

The results of this research suggest that the methanolic extracts of *R. pulcher* exhibit significant antinociceptive activity in both central and peripheral regions. The aforementioned extracts exhibited efficacy in mitigating acute toxicity. Regarding the incidence of toxicity, it was observed that MERP exhibited a higher degree of toxicity at elevated dosages. Based on a toxicity classification, it can be inferred that the aforementioned extracts possess a relatively high level of toxicity (Hossein *et al*., 2002) [28].

4. Conclusion

Antioxidant, antibacterial, cytotoxic, antinociceptive, neuropharmacological, and acute toxicity effects have all been observed in the methanolic extract of *Rumex pulcher*. Antioxidant tests showed that this plant was more effective than others. There was a little antibacterial effect with MERP. High cytotoxicity was found in the study from the bioassay on the lethality of brine shrimp. The antinociceptive action of this plant has been shown in the writhing test and the hot plate test. In addition, it was shown that a higher dose is superior than a lower one. The plant significantly affects the brain and nerve system. Effects on the central nervous system have been investigated and published in a small number of publications. The acute toxicity data establish this plant as hazardous.

5. Acknowledgement

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6. Ethical Approval

The authors confirm that all experiments were conducted in accordance with ethical guidelines and were reviewed by an appropriate ethics committee.

7. Competing interests

Authors have declared that no competing interests exist.

References

1. Banerjee A, Dasgupta N, De B. *In vitro* study of antioxidant activity of Syzygium cumini fruit. Food Chemistry. 2005;90(4):727-733. https://doi.org/10.1016/j.foodchem.2004.04.033
2. Belofsky G, Aronica M, Foss E, Diamond J, Santana F, Darley J, *et al*. Antimicrobial and antiinsectan phenolic metabolites of dalea searlsiae. Journal of Natural Products. 2014;77(5):1140-1149. https://doi.org/10.1021/np401083g

3. Bunman S. (n.d.). Study the Effect of Cannabidiol Topical on Antinociceptive and Anti-inflammatory Activities in Animal Model. 1–14.
4. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. Pain. 1985;22(1):1-31. https://doi.org/10.1016/0304-3959(85)90145-9
5. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta - General Subjects. 2013;1830(6):3670-3695. https://doi.org/10.1016/j.bbagen.2013.02.008
6. Dash PR, Mahmuda N, Rani SM. Evaluation of Analgesic and Neuropharmacological Activities of. Int. J. Pharm Sci Res. 2015;2(4):979-984. https://doi.org/10.13040/IJPSR.0975-8232.2(4).979-84
7. Franzotti EM, Santos CVF, Rodrigues HMSL, Mourão RHV, Andrade MR, Antoniolli AR. Anti-inflammatory, analgesic activity and acute toxicity of *Sida cordifolia* L. (Malva-branca). Journal of Ethnopharmacology. 2000;72(1–2):273-277. https://doi.org/10.1016/S0378-8741(00)00205-1
8. Genelyn G, Madjos AML. Comparative Cytotoxic Properties of Two Varieties of Carica papaya leaf extracts from Mindanao, Philippines using Brine Shrimp Lethality Assay. Bulletin of Environment, Pharmacology and Life Sciences. 2019;8:113-118.
9. Habib M, Billah M, Nawrin K, Hasan M, Khalil M, Rahman M. Assessment of Analgesic and Neuropharmacological Activity of Different Extracts of *Euphorbia hirta* (Linn.) Leaf. British Journal of Pharmaceutical Research. 2016;9(2):1-7. https://doi.org/10.9734/bjpr/2016/21838
10. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M, Sohraby M, Shahlari N, *et al*. Russian olive (*Elaeagnus angustifolia* L.): From a variety of traditional medicinal applications to its novel roles as active antioxidant, anti-inflammatory, anti-mutagenic and analgesic agent. Journal of Traditional and Complementary Medicine. 2017;7(1):24-29. https://doi.org/10.1016/j.jtcme.2015.09.004
11. Gangbé L, Dessouassi E, Houndonougbo PK, Brahim AA, Djimènou D, Agadjihouedé H, *et al*. Trophic regime of the giant freshwater shrimp *Macrobrachium vollenhoveni* (Herklots, 1857) in the lower Oueme valley in Southern Benin. Int. J Biol. Sci. 2022;4(1):37-45. DOI: 10.33545/26649926.2022.v4.i1a.52
12. Imam MZ, Sultana S, Akter S. Antinociceptive, antidiarrheal, and neuropharmacological activities of *Barringtonia acutangula*. Pharmaceutical Biology. 2012;50(9):1078-1084. https://doi.org/10.3109/13880209.2012.656850
13. Khaliq T, Akhter S, Sultan P, Hassan QP. Critical review on *Rumex dentatus* L. a strong pharmacophore and the future medicine: Pharmacology, phytochemical analysis and traditional uses. Heliyon, 2023, e14159. https://doi.org/10.1016/j.heliyon.2023.e14159
14. Lagarto Parra A, Silva Yhebra R, Guerra Sardiñas I, Iglesias Buela L. Comparative study of the assay of *Artemia salina* L. And the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Phytomedicine. 2001;8(5):395-400. https://doi.org/10.1078/0944-7113-00044
15. Li JJ, Li YX, Li N, Zhu HT, Wang D, Zhang YJ. The genus Rumex (Polygonaceae): an ethnobotanical, phytochemical and pharmacological review. Natural Products and Bioprospecting, 2022, 12(1). https://doi.org/10.1007/s13659-022-00346-z
16. Los UMDECDE. (n.d.). No 主観的健康感を中心とした在宅高齢者における健康関連指標に関する共分散構造分析Title.
17. Matthews RS. *Artemia salina* as a test organism for measuring superoxide-mediated toxicity. Free Radical Biology and Medicine. 1995;18(5):919-922. https://doi.org/10.1016/0891-5849(94)00205-X
18. Naz R, Bano A. Phytochemical screening, antioxidants and antimicrobial potential of Lantana camara in different solvents. Asian Pacific Journal of Tropical Disease. 2013;3(6):480-486. https://doi.org/10.1016/S2222-1808(13)60104-8
19. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs from 1981 to 2014. Journal of Natural Products. 2016;79(3):629-661. https://doi.org/10.1021/acs.jnatprod.5b01055
20. Pal S, Sen T, Chaudhuri AKN. Neuropsycho-pharmacological Profile of the Methanolic Fraction of Bryophyllum pinnatum Leaf Extract. Journal of Pharmacy and Pharmacology. 2010;51(3):313-318. https://doi.org/10.1211/0022357991772312
21. Raycheva T, Temsch EM, Dimitrova D. *Rumex pulcher* (Polygonaceae) in the Bulgarian flora: distribution, morphology, and karyology. Phytol. Balcan. 2007;13(3):321-330.
22. Santos CC de MP, Salvadori MS, Mota VG, Costa LM, de Almeida AAC, de Oliveira GAL, *et al*. Antinociceptive and Antioxidant Activities of Phytol *In Vivo* and *In vitro* Models. Neuroscience Journal, 2013, 1-9. https://doi.org/10.1155/2013/949452
23. Shaira HA, Shomudro HK, Chowdhury SA. *In vitro* and *In vivo* Pharmacological Evaluation of Persicaria lapathifolia Available in Bangladesh. Journal of Scientific Research and Reports. 2023;29(3):12-26. https://doi.org/10.9734/jsrr/2023/v29i31733
24. Shajib MS, Akter S, Ahmed T, Imam MZ. Antinociceptive and neuropharmacological activities of methanol extract of *Phoenix sylvestris* fruit pulp. Frontiers in Pharmacology. 2015;6:1-12. https://doi.org/10.3389/fphar.2015.00212
25. Shomudro HK, Shaira HA, Afreen S. Evaluation of *in vitro* antioxidant, anti-bacterial, cytotoxic and *in vivo* analgesic and neuro-pharmacological investigation of alysicarpus vaginalis available in Bangladesh. 2023;12(1):316-323.
26. Sulaiman MR, Hussain MK, Zakaria ZA, Somchit MN, Moin S, Mohamad AS, *et al*. Evaluation of the antinociceptive activity of Ficus deltoidea aqueous extract. Fitoterapia. 2008;79(7-8):557-561. https://doi.org/10.1016/j.fitote.2008.06.005
27. Taskin T, Bitis L. *In vitro* antioxidant activity of eight wild edible plants in Bursa province of Turkey. Farmacia. 2016;64(5):706-711.
28. Hosseinzadeh H, Mohammad R, Masoumeh F, Masoud M. Antinociceptive, Anti-Inflammatory and Acute Toxicity Effects of *Pistacia Vera* L. Gum Extract in Mice and Rat. Phytomedicine. 2002;1(4):0-0.