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Mustakim Hossain Tawhid
Student, Department of Pharmacy, International Islamic University Chittagong, Bangladesh

Safrin Jannat
Student, Department of Pharmacy, International Islamic University Chittagong, Bangladesh

Jesmin Aktar Dina
Student, Department of Pharmacy, Stamford University Bangladesh, Bangladesh

Faria Tasnim
Student, Department of Pharmacy, International Islamic University Chittagong, Bangladesh

Anan Mahabub Aboni
Student, Department of Pharmacy, Stamford University Bangladesh, Bangladesh

Corresponding Author:
Anan Mahabub Aboni
Student, Department of Pharmacy, Stamford University Bangladesh, Bangladesh

Evaluation of anti-oxidant, anti-nociceptive, CNS depressant and antidiarrheal activities of *Rorippa palustris* leaf extract

Mustakim Hossain Tawhid, Safrin Jannat, Jesmin Aktar Dina, Faria Tasnim and Anan Mahabub Aboni

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Abstract
This study aimed to examine certain *in vitro* and *in vivo* characteristics of plant extracts obtained from *Rorippa palustris* through the use of methanol extraction. The principal mode of evaluation for the methanolic extract of *Rorippa palustris* (MERP) was established to investigate the plant's *in vitro* potential for activities such as antioxidant, antinociceptive, neuropharmacological, and antidiarrheal activity. This is due to the pharmaceutical interest in the constituent parts of the plant. The DPPH Free Radical Scavenging Assay was utilized to measure antioxidant activity across multiple doses. The hot plate method, acetic-acid-induced writhing test, and glutamate-induced mechanism test were employed to conduct *in vivo* antinociceptive testing. The study conducted a neuropharmacological experiment utilizing the hole cross and open field methods, as well as an anti-diarrheal test employing the magnesium sulfate-induced diarrheal test. The IC_{50} value of 160.04 µg/mL has demonstrated the antioxidant activity of MERP, as per the results of the conducted research. The results of the hot plate test and the acetic acid-induced writhing test indicated a noteworthy antinociceptive effect, with a percentage of inhibition of 46.2% and 66.61% observed at doses of 200 and 400 mg/kg, respectively. The assessment of the antinociceptive activity mechanism (s) of the glutamate-induced writhing test has yielded noteworthy outcomes, with a substantial percentage of inhibition recorded at 52.35% and 75.93%. The neuropharmacological examination has exhibited potential efficacy, while the antidiarrheal evaluation has demonstrated 80.15% and 85.00% inhibition rates. In conclusion, it is evident that the phytochemical present in this plant exhibits potent pharmacological properties, making it a promising candidate for drug discovery in various fields.

Keywords: *Rorippa palustris*, antioxidant, antinociceptive, neuropharmacological, and antidiarrheal

1. Introduction
Traditional medicine's contributions to the pharmaceutical industry and drug development have been substantial since they have supplied a wealth of therapeutic compounds. Both Ayurveda and traditional Chinese medicine (TCM) have a long history of employing natural medicines to address a wide range of health issues. These traditional treatments have undergone extensive investigation, leading to the development of several medications employing their active components (World Health Organization [WHO], 2019). The anti-malarial medication artemisinin, for instance, is derived from the traditional Chinese medicine plant *Artemisia annua*, which is also used to treat malaria (WHO, 2019). Similar to how the invention of the medicine reserpine, which is used to treat hypertension and mental health conditions, may be traced back to an Indian medicinal plant, *Rauwolfia serpentina* (WHO, 2019). Furthermore, medications for disorders like osteoarthritis have been developed using molecules from the Ayurvedic plant *Boswellia serrata* because of its significant anti-inflammatory characteristics [1].
The perennial flowering plant *Rorippa palustris*, frequently referred to as marsh yellowcress or bog yellowcress, is a member of the Brassicaceae family. It may be found in numerous parts of North America, Europe, and Asia and is a native of wetland environments including marshes, bogs, and stream banks. The plant often forms thick clusters and develops to a height of between 12 and 24 inches (or 30 to 60 cm). It has upright stems with lance-shaped, dark green leaves that are pinnately split. Along the stem, the leaves are arranged alternately and have serrated edges. During the spring and summer, marsh yellowcress blooms in thick racemes of vivid yellow flowers. Small in size, the blooms have four petals and six stamens [2]. Additionally, *Rorippa palustris* has been used in conventional herbal treatment.

It includes many bioactive substances that may have therapeutic benefits, including flavonoids, phenolic acids, and glucosinolates. Marsh yellowcress has a long history of usage in traditional medicine, including the treatment of scurvy, liver problems, digestive issues, and as a diuretic and blood cleanser [3]. Even though *Rorippa palustris* has a long history of traditional usage, it is vital to get the advice of a doctor or botanist before using it as a medicine since accurate identification and dose are required. Additionally, it's critical to guarantee that the plant is collected from pristine, unpolluted settings.

This investigation is designed to determine the existence of an extract made with methanol from this plant have antioxidant, antinociceptive, neuropharmacological, and antidiarrheal properties.

2. Materials and Method

2.1 Plant sample

A specimen of *Rorippa palustris* was procured from West-Banasree, Dhaka in the month of February 2023. The botanical experts at the Department of Botany, located in Shahbag of Dhaka, have accurately recognized the plant. This department is affiliated with the University of Dhaka. The entire plant was subjected to a 14-day shade-drying process before being finely ground into powder for research purposes.

2.2 Reagents

The aforementioned chemicals, namely methanol, sodium hydroxide, diluted hydrochloric acid, concentrated sulfuric acid, and acetic acid, were supplied by Sigma Chemical Co. located in the United States of America. The sterile saline solution was procured via Orion Infusion Ltd. Square Pharmaceuticals Ltd. manufactured Diazepam and diclofenac sodium injections. The DMSO was sourced from the German enterprise Merck. Morphine sulphate was supplied by Gazi Pharmaceuticals Ltd., while Castor oil was procured from a local vendor in Dhaka.

2.3 Preparation of plant sample

The cold maceration technique was employed for the extraction process [4]. The crude extract was obtained by filtering the liquid resulting from the soaking of 695g of plant leaf powder in 900 mL of ethanol for a period of 13 days. Following filtration, the solution underwent a subsequent air-drying process for a duration of nine days. Upon completion of the procedure, the cumulative mass of crude extracts amounted to 33.5g.

2.4 Antioxidant test

2.4.1 DPPH radical scavenging activity

The methodology employed by Rao *et. al*. was utilised to assess the antioxidant activity of the specimens [5]. The assessment was carried out based on the capacity of the samples to scavenge the stable DPPH free radical. The sample was subjected to various concentrations (500, 250, 125, 62.5, 31.25, 15.62, and 7.125 µg/mL) for analysis. A volume of 1 mL for each concentration was introduced into a 3 mL solution of 0.1 mmol/L of methanol containing DPPH. Following 30 minutes of observation conducted under dark environments, the absorbance was measured at a wavelength of 517 nm and the percentage of inhibitory activity was computed using the formula:

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

2.5 Experimental Animals

The study utilized male Swiss albino mice with a weight range of 20-25 g, which were procured from the animal breeding laboratories of Jahangirnagar University located in Dhaka. The animals were subjected to a 5-day acclimatization period to adjust to the conditions of the animal room. The subjects were sustained on a conventional pellet diet and provided with unrestricted access to water. The participants were provided with unrestricted access to water, while the provision of food was discontinued one day before the commencement of the experiment. Each group was comprised of no less than five animals. During the course of the experiments, the animals were handled in accordance with the recommended ethical protocols for the proper treatment of laboratory animals.

2.6 Anti-nociceptive test

2.6.1 Hotplate test

The research investigation utilized the hot plate test to measure response latencies, following the methodology outlined by Sulaiman *et al*. The experimental setup utilized a Ugo Basile model-7280 hot plate, with a temperature of 50±2 °C. The study quantified response latency by recording the duration between the placement of an animal in the Perspex cylinder on the heated surface and the initiation of either rear paw licking or leaping behaviour. The administration of MERP (250 and 500 mg/kg, I.P.) and morphine (5 mg/kg, I.P.) occurred 30 minutes before the commencement of the experiment. The mice were subjected to pre-treatment observation, followed by post-treatment monitoring at intervals of 30, 60, 120 and 180 minutes. According to Sulaiman *et al*., the maximum duration was 20 seconds [6].

$$\text{Percent Analgesic Score} = \frac{Ta - Tb}{Ta} \times 100.$$

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

2.6.2 Acetic acid-induced writhing test

The present study employed the methodology outlined by Shomudro, Shaira *et al*. with slight adaptations to investigate the potential analgesic properties of the plant extract [7]. Each group consisted of 5 mice that were administered either diclofenac sodium (at a dosage of 10 mg/kg), plant extract (at dosages of 250 and 500 mg/kg), or normal saline solution (at a dosage of 10 mL/kg). The control group was administered with a placebo saline solution. Following the segregation of the animals into their respective cohorts, an intraperitoneal (I.P.) injection of 0.7% acetic acid (10 mL/kg) was administered. Following the administration of acetic acid, the frequency of abdominal constrictions, also known as writhes, that transpired within the 5 to 15-minute interval was recorded. The observation that tests animals exhibited reduced writhing compared to the control group animals was interpreted as indicative of an analgesic response.

$$\% \text{ of writhing} = \frac{VC - VT}{VC} \times 100$$

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

2.7 Investigation for antinociceptive activity mechanism(s)

To investigate the potential of MERP to modulate the glutamatergic pathway and exhibit antinociceptive effects,

animal subjects were administered with one of three substances: Vehicle (0.2% tween 20, orally), diclofenac sodium (10 mg/kg B.W., intraperitoneally), or MERP (50, 100, or 200 mg/kg B.W., orally). This experimental design was employed in studies conducted by Ani *et al*. (2016) [8]. 10 µM of glutamate was administered into the sub-plantar area of the right hind paw, 30 minutes following vehicle or MEBS administration and 15 minutes following standard drug administration. Following the injection, the duration of paw licking and biting was observed for a period of 15 minutes. Subsequently, the percentages of nociception inhibition were computed utilizing the aforementioned methodology [8].

2.8 Neuropharmacological test
2.8.1 Hole cross test
The cage dimensions of 0.30 m x 0.20 m x 0.14 m were established utilizing a method developed by Shaira and Shomudro [9]. A partition was erected bisecting the interior space. A circular aperture measuring 0.03 m in diameter was excavated at an elevation of 0.075 m within the central portion of the edifice. The subjects of the experiment were segregated within a specific region of the enclosure and administered one of three substances: a placebo, a reference specimen, or a test specimen. The study involved quantifying the count of mice traversing the entrance between two chambers over a period of 5 minutes at 30, 60, 90, and 120-minute intervals after the administration of control, standard, and experimental extracts. Group II was administered the gold standard medication, diazepam, while Group I was given a placebo in the form of distilled water. In the study conducted by Shaira *et al*. (2023) [9], the administration of MERP was carried out orally at a dosage of 250 mg/kg and 500 mg/kg of body weight for Groups III and IV, respectively.

2.8.2 Open field test
Ripa *et al*. detailed procedures were used for the experiments. A number of one-meter squares were tiled into the open space. The entire thing was enclosed by a wall that was about 40 centimetres high. Mice were given an oral test drug treatment of MERP and an intraperitoneal dose of Diazepam, and the number of squares they visited for 5 minutes was recorded at 30, 60, 90, and 120 minutes [10].

2.9 Anti-diarrheal test
2.9.1 Magnesium sulfate-induced diarrhoea in mice
Before the experiment, mice of both genders were subjected to a fasting period of 18 hours. The experiment was conducted in accordance with established protocols [11]. The experimental animals were segregated into eleven cohorts, each consisting of six mice. The first group, designated as the control group, was administered a vehicle comprising 1% Tween-80 in normal saline at a dose of 5 mL/kg. The second group, referred to as the positive control group, was administered loperamide orally at a dose of 3 mg/kg body weight, which served as the standard drug. The experimental groups, namely groups 3, 4, and 5, were administered with aqueous extract, while groups 6, 7, and 8 received methanol extract, and groups 9, 10, and 11 were given DCM extracts orally at a dose of 50, 100, and 200 mg/kg body weight, respectively. After an hour, each mouse was subjected to oral administration of magnesium sulfate at a dosage of 2 g/kg. The specimens were individually confined within enclosures situated atop sheets of white filter paper. The frequency of defecation with loose or unformed stools was documented over a duration of 240 minutes [12].

$$\% \text{ of inhibition} = \frac{No - Ns}{No} \times 100$$

Where, the variables "No" and "Ns" represent the mean number of faecal occurrences in the control and treatment/ standard groups, respectively.

2.10 Statical analysis
The outcomes of all bioassays were documented in triplicate, and the information presented in the table denotes the mean value. The statistical analysis was conducted utilizing Microsoft Excel.

3. Results and Discussions
3.1 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. According to Banerjee *et al*. (2005) [13], the process of neutralizing DPPH involves the provision of hydrogen or an electron by antioxidants [13]. Table 1 illustrates the ability of the methanolic extract of *Rorippa palustris* to scavenge DPPH radicals. In comparison to the control butylated hydroxyl toluene (BHT), the extract's DPPH radical-scavenging activity varied from 8.61 to 84.14%. The augmented quantity of phenolic or flavonoid bio-actives obtained through a solvent with relatively high polarity could potentially account for the amplified DPPH scavenging activity observed in the methanolic extract of *Rorippa palustris* 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	8.61	160.04
	15.625	18.88	
	31.25	36.11	
	62.5	66.38	
	125	84.44	
	250	84.14	
	500	84.14	

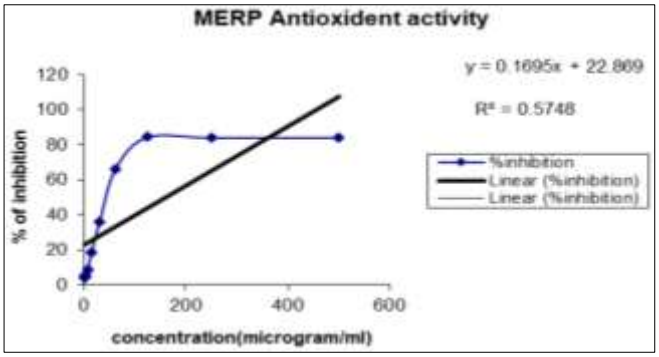

Fig 1: Antioxidant activity of MERP

3.2 Antinociception test
3.2.1 Hot plate test
Table 2 displays the findings of the investigations that were carried out using the hot plate. After oral administration of MERP at doses of both 200 and 400 mg/kg, the latency time (In seconds) was significantly extended as compared to the group that acted as the control. This was seen in comparison

to the group that received no MERP. Both of the research groups had a dose-dependent extension of the delay time that lasted longer. When given at a dosage of 10 mg/kg, morphine had the highest potential to lengthen the latency period. The extracts revealed their full potential in terms of the impacts they had after 90 and 120 minutes after being administered.

Table 2: 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	20 to 26	6.4	7.6	6.0	5.4
Morphine (5 mg/kg)		8.6	9.4	11.0	6.6
MERP (200 mg/kg)		9.6	12.4	14.8	0.0
MERP (400 mg/kg)		12.2	13	13.2	0.0

The hot plate test is employed for the evaluation of centrally-acting analgesics [14]. According to previous research, the intricate behaviours of mice, such as paw-licking or leaping, in response to hot plate stimuli involve complex supraspinal coordination. A decrease in licking or an increase in latency is indicative of the centrally-acting analgesic properties of the medication [15]. The results of the hot plate test indicate that the extracts of *Rorippa palustris* exhibit antinociceptive properties against heat-induced discomfort. The observable effect was evidenced by the elongation of the latency period up to the fifth observation, which occurred at the 120-minute mark.

3.2.2 Acetic acid-induced writhing test

Table 2 displays the significant reduction in the writhing response to acetic acid across all dosages through the administration of conventional medication (diclofenac sodium) and MERP. At a dose of 400 mg/kg, MERP exhibited the highest percentage of writhing inhibition (66.61%) compared to all other tested dosages. The highest standard medication (diclofenac sodium) demonstrated a maximum percentage inhibition of 79.61%.

Table 3: Acetic acid-induced writhing test on mice using MERP

Administered Substance	Dose	% Writhing	% of Inhibition
Control	10 mL/kg	100	0.00
Diclofenac sodium	10 mg/kg	20.40	79.61
MERP	200 mg/kg	53.80	46.2
MERP	400 mg/kg	33.39	66.61

The acetic acid-induced writhing test is a widely accepted method for evaluating the effectiveness of local and systemic analgesics in reducing pain sensitivity. 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 induction of pain expressing abdominal constriction is caused by the activation of major afferent nociceptors in the central nervous system through systemic inflammatory mediators, as stated by Santos *et al*. (2013). Table 3 illustrates a significant reduction in the occurrence of abdominal constrictions induced by acetic acid in mice following the oral administration of MERP. The results indicate that the antinociceptive effects of MERP are attributed to its inhibition of COX, LOX, and other endogenous inflammatory mediators, as well as the signal transduction originating from primary afferent nociceptors. There is evidence that central and peripheral pathways contribute to phytol's antinociceptive effects [16].

3.2.3 Investigation for antinociceptive activity mechanism (s)

The result from the glutamate-induced nociception shows that oral administration of MERP (200,400 mg/kg) produced dose-dependent inhibition of the glutamate-induced nociception. Diclofenac sodium (10 mg/kg) was used as positive control drug and showed similar significant inhibition as compared to the control group. All treatments showed significant antinociceptive activity compared with the control group.

Table 4: Effect of MERP on glutamate-induced nociception.

Treatment	Dose (mg/kg)	Licking time (s)	% of inhibition
Control	0.1 ml/mouse	26.00	0
Diclofenac sodium	10	4.60	82.30
MERP	200	12.40	52.35
MERP	400	6.00	75.93

3.3 Neuropharmacological activity

3.3.1 Whole Cross Method

Table 4 indicates a notable reduction in locomotor activity during the whole cross-experiment for the 200 mg/kg and 400 mg/kg body weight dosages in comparison to the control group. The research findings indicate that the most significant reduction in movement occurred at 90- and 120-minutes post-drug administration.

Table 5: The primary data table for the open field test for the MERP

Number of movements (Mean value)					
Group	**Average wt. of mice (g)**	**30 min**	**60 min**	**90 min**	**120 min**
Control	22 to 28	23.8	24.4	23.8	19.4
Diazepam		9.67	6.67	3.67	2.33
MERP (200 mg/kg)		10.7	9.2	6.4	0.87
MERP (400 mg/kg)		9.5	5.2	3.2	0.00

Moreover, given that the aforementioned extract inhibited both peripheral and central pain mechanisms, it is conceivable that the extract engaged with opioid receptors, as suggested by Pal *et al*. [17]. The neuropharmacological properties of MERP were assessed and it was observed that the plant extract exerts an effect on the central nervous system. According to Dash *et al*., the research findings demonstrate that mice exhibit a depressant activity, as evidenced by a reduction in their exploratory behaviour [18].

3.3.4 Open field test

The research revealed that the most significant decline in locomotion was observed at the 90 and 120-minute marks post-drug delivery. The results of the open field test indicate that the administration of both doses of the extract (200 mg/kg and 400 mg/kg) resulted in a significant reduction in the number of squares travelled by the mice, as compared to their initial score. The observed effect exhibited a level of similarity to that of the conventional pharmaceutical Diazepam, as presented in Table 5. The results indicate that the highest degree of suppression occurred at 90- and 120-

minutes post-drug administration.

Table 6: 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 (200 mg/kg)		72.6	53.6	38.4	24.2
MEPLL (400 mg/kg)		57.6	35.2	20.2	0.0

The results of this investigation suggest that the extract derived from *Rorippa palustris* demonstrates significant analgesic and central nervous system depressant effects. The findings of this study provide corroboration for the conventional therapeutic application of the aforementioned plant in the management of ailments linked to inflammatory processes [19].

3.4 Anti-diarrheal activity

Table 6 displays the outcomes of the impact of *Rorippa palustris* on diarrhoea induced by magnesium sulfate. At the concentrations of 200 and 400 mg/kg, all of the extracts exhibited significant statistical inhibition of diarrhea when compared to the control group. Following a four-hour period, the administration of loperamide at a dosage of 10 mg/kg resulted in a respective inhibition of diarrhea by 80% and 84.98%. The values corresponding to the Minimum Effective mice Dose (MERP) are 400 mg/kg each, with a respective 70, 25%. The potency of the observed order of inhibition of defecation and diarrhea is significant.

Table 7: Antidiarrheal effects of *Rorippa palustris*

Administered Substance	Dose	% inhibition of defection	% of Inhibition of diarrhea
Control	10 mL/kg	0.00	0.00
Loperamide	10 mg/kg	80.21	84.97
MERP	200 mg/kg	40.31	80.13
MERP	400 mg/kg	60.74	85.00

The extracts exhibited significant antidiarrheal activity. Magnesium sulfate induces the secretion of cholecystokinin from the mucosa of the duodenum and enhances peristaltic activity [20]. Consequently, this leads to a decrease in the gastrointestinal tract's capacity to absorb water and sodium, ultimately resulting in the manifestation of diarrhoea. The administration of *Rorippa palustris* extracts before the induction of diarrhea by magnesium sulfate resulted in a significant reduction of the symptom.

4. Conclusion

To put it briefly, *Rorippa palustris*, which is colloquially referred to as marsh yellowcress, displays encouraging antioxidant, antinociceptive, neuropharmacological, and anti-diarrheal properties. The antioxidant properties of the plant are essential in mitigating oxidative stress, a contributing factor to a range of illnesses and ailments. The observed antinociceptive activity of the substance indicates its potential as a natural analgesic agent, which may offer a remedy for pain and discomfort. Additionally, *Rorippa palustris* exhibits neuropharmacological properties, suggesting its capacity to regulate the central nervous system and provide therapeutic advantages for neurological ailments. Finally, the anti-diarrheal property of the plant suggests its potential to mitigate gastrointestinal discomfort and enhance digestive well-being. Additional investigation and examination of the phytochemical components and modes of operation of *Rorippa palustris* are necessary to comprehensively comprehend its medicinal capabilities and establish innovative therapies originating from this flora. In general, *Rorippa palustris* exhibits significant potential as a valuable reservoir of natural compounds possessing varied pharmacological properties, rendering it a captivating contender for prospective drug exploration and advancement.

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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

The authors have declared that no competing interests exist.

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