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Pharmacological Spectrum of *Boerhavia repens* Fractions: *In Vitro* and *In Vivo* Insights into Antioxidant, Anti-Inflammatory, Analgesic, CNS Depressant and Non-Cytotoxic Potentials

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Abstract

This study comprehensively investigated *Boerhavia repens* (Family: Nyctaginaceae) to assess its biological potentials. Hexane (BRH), chloroform (BRC), ethyl acetate (BRE) and aqueous (BRA) fractions were obtained by fractionating the crude methanolic extract (BRX) using the modified Kupchan method. Antioxidant, anti-inflammatory and cytotoxic properties were investigated *in vitro*, while analgesic, anti-diarrheal and CNS depressive effects were evaluated *in vivo* using mouse models. BRE demonstrated significant antioxidant activity (IC_{50} = 1.38 µg/ml), outperforming standard BHT (1.75 µg/ml) in free radical scavenging assay and yielded total phenolic content (4.29 mg GAE/g extract), in contrast to gallic acid. Compared to standard acetylsalicylic acid (88.67% and 89.91%), BRH showed notable anti-inflammatory effects (79.64% and 61.27% inhibition) in hemolysis induced by temperature and hypotonic solution. On Vero cells, no fractions showed any signs of cytotoxicity. BRX-400 (400 mg/kg) had significant dose-dependent analgesia with a pain inhibition of 63.75% contrasting to that of standard diclofenac (66.49%) in acetic acid-induced writhing test. In the formalin test, BRX-400 exhibited greater analgesic efficacy in the chronic phase (70.41%) than in the acute phase (50.58%), while diclofenac produced 83.70% and 94.91% inhibition, respectively. Similar to diclofenac (55.67–89.0%), BRX-400 retained sustained analgesia (51.67–82.0%) in the hot plate test and showed comparable outcomes in the tail immersion test (26.18–58.86% vs 22.40–52.21%, respectively). The fractions showed no discernible antidiarrheal effect. Strong, dose-dependent CNS depression was demonstrated by the extracts; in thiopentalinduced sleep and hole-cross tests, BRX-400 presented 254.98% and 45.83–84.99% effect, respectively, comparable to standard diazepam (265.92% and 20.83–79.72%). All data were statistically significant ($p<0.05$).

Key words: *Boerhavia repens*, biological activities, antioxidant, anti-inflammatory, analgesic, CNS depression.

Introduction

The World Health Organization (WHO) reports that over 25% of contemporary drugs are derived from plant-based chemicals initially employed in conventional medicines. Natural and herbal remedies have become increasingly popular worldwide in recent years due to the potential adverse effects of modern drugs. According to Najmi *et al.*, 2022, natural products account for 35% of the world-pharmaceutical market, with 25% coming from plants.

Plant secondary metabolites are structurally complex compounds derived from natural sources that are generally more physiologically compatible than synthesized drugs (Thomford *et al.,* 2018). Thanks to their phytochemical components, which include alkaloids, tannins, flavonoids and other phenolic compounds (Phan *et al.,* 2001), they have

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made a substantial contribution to the development of effective antibacterial, antifungal, antiviral, antimalarial, cardioprotective and antineoplastic agents (Newman *et al.,* 2000).

The Nyctaginaceae family, known as the "four o'clock" family, includes *Boerhavia repens*, frequently found in tropical and subtropical areas such as North Australia, Africa, Taiwan, Madagascar, Hawaii and India. Various parts of *B. repens* exhibit emetic, diuretic, laxative, anti-inflammatory, antioxidant and hepatoprotective activities (Khanpara and Vaishnav, 2022). It is also believed to have antiviral and blood-purifying properties and is used in traditional Indian medicine to treat ailments like fever, constipation and jaundice (Bhosle *et al.,* 2009).

Boerharotenoids A and B (Nazir *et al.,* 2011), Eupalitin-3-O-β-D-galactopyranoside (Pandey et al., 2004), Boeravinone F (Yi-Fen *et al.,* 2002), Boeravinone (Yang *et al.,* 2001) and 5,7,3' Trihydroxycoumaronochromone (Ferrari *et al.,* 1991) are among the several phytoconstituents found in *B. repens.* Flavonoids, rotenoids, phenolic compounds and reported alkaloids and phytosterols are the main chemical groups identified in this plant, suggesting a varied phytochemical profile.

Due to the evolving evidence of the presence of crucial plant secondary metabolites in *B. repens*, this study intends to examine the antioxidant, anti-inflammatory, and cytotoxic activity of the various solvent extracts of the whole plant through *in vitro* analysis. Additionally, *in vivo* experiments on a mouse model are carried out to comprehend the analgesic, anti-diarrheal and CNS depressant properties of the plant's crude methanolic extract at various concentrations. The outcomes could make *B. repens* a potential therapeutic candidate with pharmacological value in the burgeoning pharmaceutical industry.

Materials and Methods

Sample plant acquisition

The plant specimen (Accession number: JUH 10310) was obtained from the Department of Botany, Jahangirnagar University in Dhaka, Bangladesh.

The whole *B. repens* plant was pulverized into coarse powder using a grinding machine (FFC-15, China), about 2100 grams of which was soaked in 10 liters of distilled methanol and stored in amber color containers. The containers were periodically shaken throughout the two months that the powder was soaking. After first passing through a cotton plug, the crude extract was filtered through Whatman filter paper (Grade 1). The filtrate was then concentrated under low pressure in a rotary evaporator at a temperature of 40–46°C until more than 75% of the solvent had evaporated. Up to 30 grams of extract were obtained by repeating the process. Following this, four separate fractions-hexane (BRH), chloroform (BRC), ethyl acetate (BRE) and aqueous (BRA)- were obtained through solvent-solvent partitioning of approximately five grams of the crude methanolic concentrated extract (BRX) of *B. repens* using the modified Kupchan method (Sikder *et al.*, 2016).

Drugs and reagents

Sigma-Aldrich, Fischer, Merck and Loba Chemie supplied the chemical reagents used in the process. Methanol, n-hexane, chloroform, ethyl acetate and other analytical grade solvents were acquired from Merck (Germany). Formalin, tween-80, loperamide (Square Pharmaceuticals PLC.), normal saline (Opsonin Pharma Ltd.), acetylsalicylic acid (Essential Drugs Company Ltd.), thiopental sodium (Gonoshasthaya Pharma Ltd.) and castor oil were purchased from the community market.

Biological investigations: *In vitro* assays

Investigation of antioxidant activity

DPPH free radical scavenging activity assay: The antioxidant potential of *B. repens* methanolic extract was evaluated using the DPPH free radical scavenging assay (Brand-Williams *et al.,* 1995). Initially, a standard solution of butylated hydroxytoluene (BHT) in methanol at a concentration of 500 µg/ml was prepared to conduct the test and serial dilution was used to produce various concentrations down to ~0.98 µg/ml. Test samples of identical concentrations were acquired by mixing 4

ml of methanol with 4 mg of *B. repens* fractions and the crude extract. A 0.1 mM DPPH solution was added to each test tube containing test materials or positive control (BHT), which were then placed in the dark for 30 minutes. The absorbance at 517 nm was measured against methanol using a UV-vis spectrophotometer. To determine the reference absorbance, a control solution comprising DPPH and methanol was made, lacking any sample or BHT. The antioxidant effect is represented using the following formula:

$$\%I = \{(A_{control}-A_{sample})/A_{control}\} \times 100$$

Where, I = Inhibition of DPPH, $A_{control}$ = Absorbance of blank, A_{sample} = Absorbance of sample

Subsequently, the IC_{50} value is determined for the standard and test samples.

Total phenolic content (TPC): Gallic acid was used as a standard in this colorimetric analysis, which followed Škerget's methods (Škerget *et al.,* 2005). The gallic acid standard was serially diluted to ~0.98 µg/ml from 125 µg/ml. A solution of 7.5% Na_2CO_3 and test samples at concentrations of 2 mg/ml in distilled water were prepared. Folin-Ciocalteu reagent (2N) was diluted 20 times; 0.5 ml of each standard or sample was combined with 2.5 ml of the diluted reagent and 2 ml of Na_2CO_3. After 20 minutes, absorbance measurements at 760 nm generated a standard curve for calculating the total phenolic content as mg gallic acid equivalents (GAE)/g extract (Figure 1).

Investigation of anti-inflammatory activity

Membrane-stabilizing effect: The present investigation used the method described by Shinde *et al.* as a study protocol to assess the anti-inflammatory qualities of extract fractions *in vitro* from the *B. repens* plant (Shinde *et al.,* 1999). In this work, human erythrocytes were hemolyzed by heat and hypotonic stress using acetylsalicylic acid as a standard.

Investigation of cytotoxic activity

Test for cytotoxic activity using the non-cancerous Vero cell line: In an effort to determine *B. repens*'s potential to harm healthy, growing cells, the procedure developed by Vijayarathna and Sasidharan (2012) is applied in the study. DMEM treated with 10% FBS, 2% gentamycin and 1% penicillin-streptomycin was used to culture Vero cells. 48-well plates were seeded with cells (3×10^4/200 µl) and incubated at 37°C for 24 hours. Following the addition of filtered BRH, BRC, BRE, BRA and BRX samples (50 µl; 1000 µg/ml), cytotoxicity was assessed under a microscope after a 24-hour incubation period.

Biological investigations: *In vivo* assays

Test animals

Swiss Albino mice (20–25 g) of either sex, aged 4–5 weeks, were obtained from Jahangirnagar University's animal house. The mice were kept in standardized polypropylene cages at the Institute of Nutrition and Food Science (INFS) at room temperature (24 ± 2ºC) and relative humidity (60–70%) with a 12-hour light/dark cycle. The animals were fed an icddr,b (International Centre for Diarrhoeal Disease Research, Bangladesh)-formulated meal and water ad libitum; only water was administered twelve hours prior to the trial. The research utilized as few animals as feasible while adhering to the ethical standards and guidelines of the Swiss Academy of Medical Sciences (Charan and Kantharia, 2013). For each test, five groups of six mice each were formed by random selection.

Investigation of analgesic activity

Acetic acid-induced writhing test: The acetic acid-induced writhing method was used to assess the samples' peripheral analgesic efficacy in mice (Koster *et al.,* 1959). A solution of 0.7% acetic acid was prepared for this test. Group I functioned as the negative control (0.25 ml of Tween 80 in normal saline, p.o.), Group II as the positive control (0.25 ml of standard diclofenac sodium at 10 mg/kg, p.o.) and Groups III–V were administered 0.25 ml of methanol extract (BRX) at doses of 100, 200 and 400 mg/kg, p.o., respectively. All groups were given an intraperitoneal dose of acetic acid after 30 minutes, and writhing was noted for 15 minutes, five minutes post-injection. The frequency of writhing in the

treated groups was compared to that of the control and standard groups.

Formalin-induced licking test: The study conformed to Demsie *et al*., (2019). Groups I and II were given tween 80 in saline (0.25 ml, p.o.) and diclofenac sodium (10 mg/kg, 0.25 ml, p.o.), respectively, while Groups III–V received methanol extract (BRX) at doses of 100, 200, and 400 mg/kg (0.25 ml, p.o.). After 30 minutes, each mouse was provided a 20 µl injection of 2.5% formalin into its left hind paw, and the durations of acute (0–5 min) and chronic (15–30 min) biting and licking were noted. The treatment group-reaction times were determined and contrasted with those of the standard and control groups using the formula:

% Analgesic activity = $\{(C-T)/C\} \times 100$

Where, C = Mean writhing of control group, T = Mean writhing of treatment group

Hot plate test: Tween 80 in saline (0.25 ml, p.o.) and diclofenac sodium 10 mg/kg (0.25 ml, p.o.) were administered to Groups I and II, respectively; Groups III–V were given the methanol extract (BRX) at 100, 200, and 400 mg/kg (0.25 ml, p.o.), sequentially. The animals were kept on a hot plate that was sustained at 55 ± 0.5°C. At 0, 30, 60, 90 and 120 minutes, the latency to paw licking or jumping was measured (Demsie *et al*., 2019).

Tail immersion test: Similar to previous experiments, each group received a different treatment for this test: Tween 80 in saline for Group I and diclofenac sodium for Group II, both at 0.25 ml, p.o. Groups III-V were given 0.25 ml of methanol extract (BRX) at dosages of 100, 200 and 400 mg/kg orally. The tail was submerged in water at 55 ± 0.5°C, and tail withdrawal latency times following treatment were measured at 0, 30, 60, 90 and 120 minutes (Demsie *et al.,* 2019), using the formula:

% Analgesic activity = $\{(T-C)/C\} \times 100$

Where, T = Mean latency period of treatment group, C = Mean latency period of control group

The mean of 0 and 10 minutes before treatment was used to compute the baseline reaction.

Investigation of anti-diarrheal activity

Anti-diarrheal activity in castor oil-induced diarrhea: This test was conducted using a modified version of the methods described by Shoba and Thomas (2001). 1% tween 80 in normal saline (0.25 ml, p.o.) and standard loperamide 3 mg/kg (0.25 ml, p.o.) were administered to Groups I and II, respectively. Oral doses of 100, 200 and 400 mg/kg of the test sample (methanol extract, BRX) were administered to Groups III–V. 30 minutes after the doses were administered, each mouse was given 1 ml of castor oil orally to cause diarrhea. One hour after the castor oil was applied, fecal output was measured every three hours. The diarrheal inhibition was calculated using the following formula:

% inhibition of diarrhea = $\{(C-T)/C\} \times 100$

Where, C = Mean number of feces of the control group, T = Mean number of feces of the treatment group

Investigation of CNS depressant activity

Thiopental-induced sleep time test: Tween 80 in normal saline (0.25 ml, p.o.) and standard diazepam 2 mg/kg (0.25 mL, i.p.) were given to Groups I and II, respectively. Methanol extract (BRX) of 100, 200, and 400 mg/kg (0.25 ml, p.o.) was provided to Groups III–V. All mice were administered 40 mg/kg of thiopental sodium intraperitoneally to induce sleep after 30 minutes. The latent period until the righting reflex was lost and the length of sleep until the reflex was recovered were recorded (Turner *et al.,* 1965). The effect was calculated using the formula below:

% Effect = $(T/C) \times 100$

Where, T = Mean duration of loss of righting reflex due to test samples, C = Mean duration of loss of righting reflex due to negative control

Hole cross analysis: Tween 80 with normal saline (0.25 ml, p.o.) and diazepam 2 mg/kg (0.25 ml, i.p.) were provided to Groups I and II, respectively. Oral extract (BRX) dosages of 100, 200 and 400 mg/kg (0.25 ml) were administered to Groups III–V, sequentially. At 0, 30, 60, 90 and 120 minutes following treatment, the number of crossings within three minutes was used to gauge locomotor activity

(Takagi et al., 1971). The % inhibition of movement was estimated using the formula:

% Inhibition= $\{(C-T)/C\} \times 100$

Where, C = Mean number of crossings in the control group, T = Mean number of crossings in the treated group

Statistical analysis

All test results were represented by the mean ± standard error of the mean (SEM), and Microsoft Excel was used to analyze the data using a one-way analysis of variance (ANOVA). At $p<0.05$, statistical data were considered significant.

Results and Discussion

The primary objective of the current investigation was to assess the organic soluble components of a methanol extract of *B. repens* for their antioxidant, membrane-stabilizing, cytotoxic, analgesic, antidiarrheal and CNS-depressing properties. Tables 1-10 provide a summary of the findings.

Evaluation of antioxidant activity

In order to assess the antioxidant capacity of *B. repens*, the IC_{50} value from the DPPH free radical scavenging activity test and the total phenolic content (TPC) value were computed.

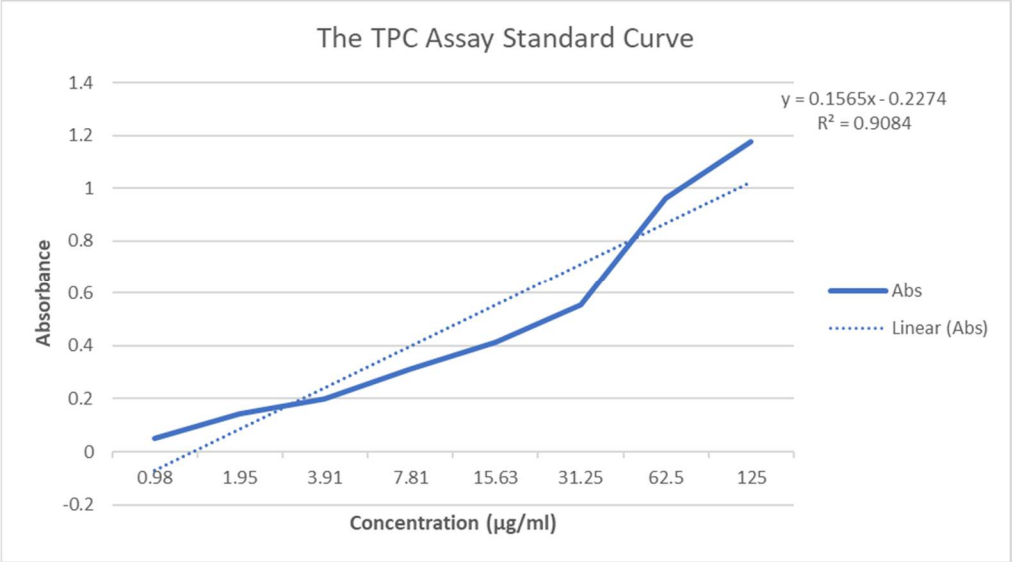
With an IC_{50} value of 1.38 µg/ml, which is considerably lower than that of the standard BHT (1.75 µg/ml), the ethyl acetate fraction of the plant (BRE) was shown to have substantial free radical scavenging activity in this assay (Table 1). In contrast, the hexane fraction (BRH) showed the least promise as an antioxidant agent (92.4 µg/ml), despite the other fractions showing moderate IC_{50} values when compared to the standard.

The total phenolic content (TPC) analysis corresponded closely with the results of the DPPH assay. A strong antioxidant activity is associated with a high phenolic concentration (Molole *et al.*, 2022). In this assay, the ethyl acetate fraction (BRE) yielded the highest total phenolic content (4.29 mg GAE/g extract), comparable to that of standard gallic acid (Table 1). The aqueous fraction (BRA) exhibited a moderate phenolic content, whereas the hexane fraction (BRH) contained the lowest, indicative of its comparatively weak antioxidant potential.

Plant phenolics offer potential anti-inflammatory, anti-cancer, cardioprotective, anti-diabetic and UV-protective effects on the skin, in addition to antifungal and antibiofilm qualities (Sun and Shahrajabian, 2023; Shahidi and Ambigaipalan, 2015). These benefits of phenolics are partly due to their antioxidant properties. In addition to scavenging free radicals, antioxidants also reduce hydroperoxides into stable hydroxyl derivatives, inactivate metal catalysts by chelation and cooperate with other reducing molecules to prevent several diseases induced by free radicals (Shahidi and Ambigaipalan, 2015).

Table 1. IC_{50} values from DPPH assay and total phenolic content (TPC) derived from standard curve.

Sample code	Antioxidant activity assays	
	IC_{50} (µg/ml)	TPC (mg GAE/g extract)
BRH	92.4	2.72
BRC	2.05	2.99
BRE	1.38	4.29
BRA	3.56	3.52
BRX	3.33	3.17
Standard BHT	1.75	



Here, BRH = Hexane soluble fraction, BRC = Chloroform soluble fraction, BRE = Ethyl acetate soluble fraction, BRA = Aqueous soluble fraction, BRX = Methanol extract, BHT = Butylated hydroxytoluene.

Figure 1. The gallic acid standard curve for total phenolic content (TPC).

Evaluation of anti-inflammatory activity by the membrane-stabilizing effect

When compared to the standard acetylsalicylic acid (0.10 mg/ml), the extractives of *B. repens* at a concentration of 2 mg/ml considerably prevented the lysis of mice RBC membranes triggered by heat and hypotonic solution. The hexane fraction of *B. repens* (BRH) exhibited a significant anti-inflammatory effect (79.64%) in a temperature-induced anti-inflammatory test, followed by the chloroform fraction (BRC) (63.33%), according to the *in vitro* experiment (Table 2). Additionally, the other extracts exhibited moderate to modest inhibition when compared to that of the standard acetylsalicylic acid (88.67%). A similar trend is seen in the hypotonic solution-induced inhibition of the inflammation test with BRH (61.27%) and BRC (52.79%) exhibiting greater inhibition than the other fractions in comparison to the inhibition by standard acetylsalicylic acid (89.91%). Aqueous fraction (BRA) was shown to have the lowest anti-inflammatory capacity in both heat-induced and hypotonic-induced RBC inhibition (42.14% and 25.13%, respectively).

Nevertheless, based on data analysis, it is reasonable to conclude that all of the components in the plant extracts have anti-inflammatory potential. Similar to how NSAIDs work, the anti-inflammatory properties of fractions of *B. repens* may be explained by the stability of lysosomal and erythrocyte membranes. This stability likely lessens tissue damage from inflammation and prevents lysosomal enzymes from being released (Ackerman and Beebe, 1974). This impact could be attributed to the extracts' flavonoids, tannins and saponins, which are known to bind cations, change membrane surface charges, and hinder hemolysis, all of which reduce inflammatory reactions (Oyedapo *et al.*, 2004; Van Caneghem, 1972; Middleton, 1996; El-Shabrawy *et al.*, 1997).

Evaluation of cytotoxicity on Vero cell lines

Based on studies obtained under a microscope, the plant *B. repens* appears to have no appreciable cytotoxic effects on non-cancerous Vero cell lines. The extract and all fractions demonstrated 5% cell death, but the blank solvent had the same impact, suggesting that 2.5% DMSO, not the samples, was the cause of the observed cytotoxicity (Table 3).

Table 2. Membrane-stabilizing effect of various *B. repens* extracts.

Sample code	% Inhibition of inflammation	
	Temperature-induced	Hypotonic solution-induced
Control	-	-
Standard	88.67	89.91
BRH	79.64	61.27
BRC	63.33	52.79
BRE	48.65	36.93
BRA	42.14	25.13
BRX	55.48	32.48

Here, BRH = Hexane soluble fraction, BRC = Chloroform soluble fraction, BRE = Ethyl acetate soluble fraction, BRA = Aqueous soluble fraction, BRX = Methanol extract, Standard = Acetylsalicylic acid, Control = Vehicle (Mixture of acacia (3%) and tragacanth (0.1% of acacia) in distilled water).

Table 3. Vero cell survival in test samples of *B. repens*.

Sample code	Survival of Vero cells
Solvent (-)	100%
Solvent (2.5% DMSO) (+)	>95%
BRH	>95%
BRC	>95%
BRE	>95%
BRA	>95%
BRX	>95%

Here, BRH = Hexane soluble fraction, BRC = Chloroform soluble fraction, BRE = Ethyl acetate soluble fraction, BRA = Aqueous soluble fraction, BRX = Methanol extract.

Evaluation of analgesic activity

In every model, *B. repens* extracts demonstrated impressive, dose-dependent analgesic effects. In the acetic acid-induced test, BRX-100 (100 mg/kg), BRX-200 (200 mg/kg) and BRX-400 (400 mg/kg) achieved inhibitions of 30.52%, 49.40% and 63.75%, respectively, which were comparable to diclofenac (66.49%) (Table 4). As reported in table 5, the extracts BRX-100, -200 and -400 demonstrated a stronger impact in the chronic phase of the formalin-induced licking test (29.06%, 45.48% and 70.41% inhibition) than in the acute phase (23.33%, 35.56%, and 50.58%), respectively, suggesting a delayed onset than standard diclofenac (94.91% and 83.70%, acute and chronic). In the hot plate test, BRX-200 had a moderate late-phase reaction (up to 50% at 120 min), but BRX-400 presented sustained activity (51.67-82%) comparable to standard diclofenac (Table 6). The tail immersion test likewise revealed that BRX-400 had the strongest analgesic effect (22.40-52.21%) with increasing level of significance in contrast to the standard, whereas lesser dosages had a minimal effect (Table 7). All results confirmed statistical significance ($p < 0.05$).

Taken together, the *B. repens* methanolic extract showed strong, dose-dependent analgesic effects. Higher doses significantly decreased pain in the acetic acid-induced writhing test, potentially through modulation of pain receptor ion channels or blocking COX-2 mRNA expression and proinflammatory cytokine synthesis (IL-33, TNF-α, IL-1β and IL-6) (Aleebrahim-Dehkordy et al., 2017; Silva-Correa et al., 2021). In the formalin-induced licking test, the extract suppressed pain in both the acute and chronic

phases, primarily impacting the latter, indicating a peripheral mechanism such as inhibition of inflammatory mediators, including prostaglandins and nitric oxide (Silva-Correa *et al.*, 2021). With longer latency durations suggesting potential participation of opioid or neurotransmitter pathways, the hot plate and tail immersion experiments provided evidence for central analgesic activity, conceivably attributed to phytochemicals such as phenolics and flavonoids (Lucarini *et al.*, 2013).

Table 4. % Analgesic effect of methanol extract of *B. repens* in acetic acid-induced writhing test.

Sample code	Number of writhing (Mean ± SEM)[a]	% Analgesic activity
Control	94.5 ± 8.14	-
Standard	31.67 ± 2.49	66.49***
BRX-100	65.66 ± 4.32	30.52**
BRX-200	47.34 ± 3.74	49.40**
BRX-400	34.25 ± 5.52	63.75***

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Diclofenac sodium, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively.

Table 5. % Analgesic effect of methanol extract of *B. repens* in formalin-induced licking test.

Sample code	Acute phase		Chronic phase	
	Licking time (Mean ± SEM)[a]	% Analgesic activity	Licking time (Mean ± SEM)[a]	% Analgesic activity
Control	72.167 ± 4.76	-	151.33 ± 11.83	-
Standard	33.67 ± 1.02	94.91***	24.67 ± 1.09	83.698***
BRX- 100	55.33 ± 4.78	23.33*	107.35 ± 3.07	29.06*
BRX- 200	46.50 ± 3.17	35.56*	82.50 ± 3.21	45.48***
BRX- 400	35.66 ± 2.23	50.58***	44.78 ± 1.78	70.41***

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Diclofenac sodium, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively.

Table 6. % Analgesic effect of methanol extract of *B. repens* in hot plate test.

Sample code	Latency period (sec) (Mean ± SEM)[a]				
	0 min	30 min	60 min	90 min	120 min
Control	2 ± 0.26	3 ± 0.26	3.33 ± 0.42	3.17 ± 0.31	3 ± 0.26
Standard	3.67 ± 0.33 (83.5**)	4.67 ± 0.42 (55.67**)	6.17 ± 0.31 (85.29***)	5.83 ± 0.54 (83.91**)	5.67 ± 0.33 (89***)
BRX- 100	2.66 ± 0.34 (33)	3.35 ± 0.24 (11.67)	3.89 ± 0.34 (16.81)	3.67 ± 0.45 (15.77)	3 ± 0.45 (0)
BRX- 200	3.12 ± 0.22 (56)	3.89 ± 0.40 (29.67)	4.73 ± 0.24 (42.04*)	4 ± 0.64 (26.18)	4.5 ± 0.45 (50*)
BRX- 400	3.55 ± 0.26 (77.5**)	4.55 ± 0.33 (51.67*)	5.23 ± 0.53 (57.06**)	5.13 ± 0.36 (61.83**)	5.46 ± 0.33 (82***)

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Diclofenac sodium, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively. The values demonstrated in parentheses represent the percentage of analgesic activity.

Table 7. % analgesic effect of methanol extract of *B. repens* in tail immersion test.

Sample code	Latency period (sec) (Mean ± SEM)[a]				
	0 min	30 min	60 min	90 min	120 min
Control	2.83 ± 0.31	3.17 ± 0.17	3.33 ± 0.21	3.67 ± 0.21	3.83 ± 0.17
Standard	4 ± 0.26 (41.34*)	4 ± 0.26 (26.18*)	4.5 ± 0.22 (35.14**)	5.83 ± 0.31 (58.86***)	6 ± 0.37 (56.66***)
BRX- 100	3 ± 0.31 (6)	3.33 ± 0.22 (5.04)	3.66 ± 0.17 (9.9)	4.11 ± 0.21 (11.98)	4.27 ± 0.48 (11.48)
BRX- 200	3.33 ± 0.28 (17.67)	3.67 ± 0.43 (15.77)	4 ± 0.11 (20.12)	4.25 ± 0.23 (15.83)	5.67 ± 0.21 (48.04**)
BRX- 400	3.67 ± 0.21 (29.68)	3.88 ± 0.36 (22.4)	4.5 ± 0.44 (35.13*)	5.17 ± 0.32 (40.87**)	5.83 ± 0.12 (52.21***)

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Diclofenac sodium, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively. The values demonstrated in parentheses represent the percentage of analgesic activity.

Evaluation of anti-diarrheal activity: Loperamide (standard) and *B. repens* extracts (100, 200 and 400 mg/kg) were among the treatment groups that exhibited lower fecal production than the control. Loperamide delivered the highest inhibition of feces (93.4%, 92.6%, 95.43% and 83.3% over 1 through 4 hours, respectively), while most *B. repens* extracts presented no significant reduction, with only a negligible, insignificant effect at 400 mg/kg (Table 8).

Evaluation of CNS depressant activity: In both the thiopental-induced sleeping and hole cross tests, the methanolic extract of *B. repens* showed strong, dose-dependent CNS depressant effect. The extract significantly extended the sleep duration in the thiopental-induced sleeping time test to 772.67 ± 13.42s (189.92%), 942.5 ± 112.56s (231.67%) and 1037.33 ± 138.86s (254.98%) at 100, 200 and 400 mg/kg, respectively, in comparison to the control (406.83 ± 70.33s) (Table 9). The highest prolongation was recorded with standard diazepam (1081.83 ± 108.59s; 265.92%). In line with this, the sleep latency dropped dramatically and significantly from 135.83 ± 9.28s in the control group to 15.67–18.83s across extracts and 19.67s with diazepam, suggesting improved sleep onset. In the hole cross test, diazepam decreased locomotor activity by 79.72-64.93% between 30 and 120 minutes, whereas the control group displayed about 12-14 crossings (Table 10). With maximum inhibitions of 70.5% (BRX-100), 73.74% (BRX-200) and 84.99% (BRX-400), the extract demonstrated a steady decline in activity that was significantly similar to the effects of diazepam. All of the results were identified as statistically significant ($p < 0.05$).

Benzodiazepines such as diazepam have sedative-hypnotic, muscle-relaxant and anxiolytic effects because of the increased activity of $GABA_A$ (gamma aminobutyric acid) (Doyno and White, 2019). Given that the data indicated that the methanolic extract of *B. repens* at different doses had an effect comparable to that of diazepam, this might provide insight into the mechanistic approach of the extract's CNS depression. Additionally, phytoconstituents have been reported to elicit neural defense from oxidative-metabolic damages; enhance nervous activity; and stimulate the transient receptor calcium channels in the nerve cell membrane, which may also play a role in demonstrating the neuro-modulatory effects (Welcome, 2020; Alzobaidi *et al.,* 2021).

Table 8. Test of *B. repens* crude methanol extract for anti-diarrheal effect.

Sample Code	Number of feces (Mean ± SEM)[a]			
	1 hr	2 hr	3 hr	4 hr
Control	5 ± 0.32	9 ± 0.4	14.67 ± 0.53	16 ± 0.52
Standard	0.33 ± 34 (93.4)***	0.67 ± 40 (92.6)***	0.67 ± 21 (95.43)***	2.67 ± 62 (83.3)***
BRX- 100	4.67 ± 0.21 (6.6)	8.67 ± 0.4 (3.67)	14.5 ± 0.21 (1.16)	15.83 ± 0.34 (1.06)
BRX- 200	4.5 ± 0.34 (10)	8.83 ± 0.62 (1.88)	14.67 ± 0.24 (0)	15.5 ± 0.34 (3.12)
BRX- 400	4.33 ± 0.23 (13.4)	7.33 ± 0.34 (18.55)	12 ± 0.4 (18.2)	14.67 ± 0.21 (8.31)

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Loperamide, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively. The values demonstrated in parentheses represent the percentage of fecal inhibition.

Table 9. % CNS depressant effect of methanol extract of *B. repens* in thiopental-induced sleep time test.

Sample code	Latent period (sec) (Mean ± SEM)[a]	Sleep period (sec) (Mean ± SEM)[a]	% Effect
Control	135.83 ± 9.28	406.83 ± 70.33	100
Standard	19.67 ± 8.09***	1081.83 ± 108.59***	265.92
BRX- 100	15.67 ± 6.93**	772.67 ± 13.42**	189.92
BRX- 200	18.83 ± 3.43***	942.5 ± 112.56***	231.67
BRX- 400	16.33 ± 4.53***	1037.33 ± 138.86***	254.98

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Diazepam, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively.

Table 10. % CNS depressant effect of methanol extract of *B. repens* in hole cross test.

Sample code	(Mean ± SEM)[a] % activity				
	0 min	30 min	60 min	90 min	120 min
Control	12 ± 0.86	12.33 ± 0.67	13.33 ± 0.84	14.17 ± 1.01	12.83 ± 0.79
Standard	9.5 ± 0.67* (20.83)	2.5 ± 0.5*** (79.72)	2.83 ± 0.44*** (78.77)	3.83 ± 0.38*** (72.97)	4.5 ± 0.42*** (64.93)
BRX-100	10.33 ± 0.34 (13.91)	4.67 ± 0.24*** (62.12)	4.5 ± 0.36*** (66.24)	4.18 ± 0.34*** (70.5)	6.83 ± 0.25*** (46.76)
BRX-200	9.33 ± 0.56** (22.25)	3.33 ± 0.2*** (72.99)	3.5 ± 0.28*** (73.74)	4 ± 0.32*** (71.77)	5.33 ± 0.52** (58.45)
BRX-400	6.5 ± 0.43*** (45.83)	2.83 ± 0.45*** (77.04)	2 ± 0.68*** (84.99)	3.89 ± 0.21*** (72.54)	5 ± 0.4*** (61.02)

[a]Each value represents Mean ± SEM, (n = 6); ***p<0.001; **p<0.01; *p<0.05 in comparison to control (One-way ANOVA); Control = Tween 80 in normal saline, Standard = Diazepam, BRX -100, 200, 400 = Methanol extract of *B. repens* at doses 100 mg/kg, 200 mg/kg, and 400 mg/kg b.w., respectively. The values demonstrated in parentheses represent the percentage inhibition of movement by test animals.

Conclusion

This study conducted a thorough investigation to evaluate the pharmacological potential of the crude methanolic extract of the whole plant *B. repens*. Subsequent solvent-based fractionation was also carried out to distinguish the activity profiles of each fraction. Neither the crude extract nor its resulting fractions demonstrated substantial antidiarrheal activity or any cytotoxicity against non-cancerous cell lines. Nonetheless, across most fractions, persistent antioxidant and anti-inflammatory properties were evident. Additionally, the plant's bioactive potential was highlighted by *in vivo* experiments in mice that showed impressive analgesic and CNS depressive actions. Given its long-standing use in traditional medicine, *Boerhavia repens* validates deeper systematic exploration to strengthen and broaden its pharmacological precedence.

Conflict of Interest

The authors affirm that they have no conflicts of interest with regard to the article's publication.

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