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Exploring the Pharmacological Potential of *Bauhinia malabarica* Roxb.: A Comprehensive In Vitro and In Vivo Investigation

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Abstract: This study investigates the potential biological effects of organic soluble fractions from the crude methanol extract of the leaf and bark of *Bauhinia malabarica* Roxb (*B. malabarica*), which is traditionally used for various ailments. Plant materials were collected, dried, and extracted using methanol. They were then extracted into aqueous fractions, carbon tetrachloride, chloroform, and petroleum-ether. The methanolic leaf extract demonstrated excellent antioxidant activity during the DPPH assay for antioxidant testing, with an IC_{50} value of 12.15 µg/mL, which is similar in efficacy to the standard ascorbic acid (IC_{50} = 5.8 µg/mL). The cytotoxicity assay was carried out using brine shrimp nauplii. All test samples of *B. malabarica* showed significant membrane-stabilizing activity as determined by the hemolysis induced by hypotonic solutions and heat. The plant samples from leaf extract and bark extract also exhibited significant anthelmintic and analgesic activity in a dose-dependent manner. These plant extracts exerted analgesic effects that were characterized by elongation of tail immersion time and reduction in abdominal writhes. The test samples reduced the quantity of diarrhea caused by castor oil in mice in a dose-dependent manner. Additionally, the extract extended the sleep duration and delayed the onset of action compared to the control group. Based on our findings, *B. malabarica* could be a rich source of bioactive compounds, meriting further exploration for its potential in traditional medicine.

Keywords: *Bauhinia malabarica*; antioxidant; cytotoxicity; membrane stabilizing; anthelmintic; analgesic; anti-diarrheal; sedative

1. Introduction

According to a 1985 estimation by the WHO, approximately 65% of the global population primarily relied on plant-derived traditional medicines for their healthcare, as they are affordable and easily accessible to the general public. Over the past 30 years, up to 50% of approved drugs have been derived directly or indirectly from natural products. Many of the medicinal substances currently in use, such as aspirin, morphine, quinine, artemisinin and taxol were obtained from plant sources [1–3]. Moreover, the use of medicinal plants in the preparation of traditional medicine has increased during the past decade. In recent years, research on plant-based medicine has emerged as an interesting area in the scientific validation of medicinal plants that are recommended by local healers and tribal societies.

Bauhinia malabarica Roxb. (Family- Leguminosae) is a small- or moderate-sized deciduous tree which is locally known as “Kanchan”. Most *Bauhinia* species are planted for their beautiful blooms and decorative shrubs [4] In Bangladesh, the plant is widely distributed in deciduous forests of Sylhet. *B. malabarica* has many medicinal properties [5]. Stems and leaves are used as diuretics, anti-infective and anti-diarrheal in traditional Thai medicine [6]. The root extracts showed in vitro antimalarial activity [7]. An infusion of the young flowers is given in dysentery. Previously, antimalarial racemosol derivatives have been extracted from the root of *B. malabarica* [8]. Decoction of this plant root was used for liver problems. Root and stem were beneficial in the treatment of cholera [9]. Flavonols were isolated from *B. malabarica* leaves [7]. The folk people in Malabar coast of Southern India

use the stem bark extract for the treatment of jaundice [10]. There are a few ethno-pharmacological surveys on the medicinal applications of *B. malabarica* [8], however there are still few laboratory data on their bioactivity. Therefore, the current study was carried out to fill these gaps by analyzing the biological and pharmacological effects of *B. malabarica* leaf and stem bark extracts utilizing established procedures.

2. Materials and Methods

2.1. Plant Material Collection and Processing

Leaves and stem bark of *B. malabarica* were collected from Romna park area, Dhaka. After collection, the taxonomical identification of the plant was completed in Bangladesh National Herbarium, Dhaka. Next, the leaves and stem barks were carefully cleaned, air-dried, and then ground into a coarse powder for further analysis.

The powdered ingredients of the *B. malabarica* leaf and stem bark were macerated separately in 200 mL of methanol for several days, allowing the plant material to completely dissolve and release its bioactive components. After maceration, the plant material was filtered using a Whatman No. 1 filter paper, and the resulting filtrate was concentrated under decreased pressure using a rotary evaporator (Buchi Rotavapor R-3) set to a temperature of 40 °C. This evaporation process removed the methanol, yielding a semisolid mass that represented the crude methanol extract of the *B. malabarica* leaf (MEL) and stem bark (MEB).

To further isolate and separate the bioactive components of the crude extracts, the modified Kupchan method [11] was employed. This method involved solvent-solvent partitioning, where approximately 5 g of each of the crude extracts (MEL and MEB) was subjected to successive extractions using 50 mL of petroleum ether, 50 mL of carbon tetrachloride, 50 mL of chloroform, and 50 mL of water. These solvent fractions were chosen based on their differing polarities, which allowed for the separation of compounds based on their solubility in each solvent.

The 5 g of crude extract effectively achieved fractionation, as it produced clear, distinct layers of soluble fractions, which were identified as petroleum-ether, carbon tetrachloride, chloroform, and water fractions (Table 1). These fractions were dried and collected separately for further analysis. The selected solvent volumes (50 mL each) ensured adequate contact between the extract and solvent, providing efficient partitioning and separation of bioactive compounds.

After fractionation, the biological activities of each of the four soluble fractions, as well as the crude extracts of leaf and bark (Table 1), were assessed separately. Through this method, we comprehensively evaluated the different bioactive components in the crude extracts and provided insights into their individual contributions to the overall biological activity.

Table 1. Test samples of *B. malabarica* used in pharmacological investigations.

Plant Part	Sample Code	Test Sample
Leaves	MEL	Methanolic soluble fraction of leaf
	PEFL	Petroleum ether soluble fraction of leaf
	CTFL	Carbon tetrachloride soluble fraction of leaf
	CHFL	Chloroform soluble fraction of leaf
	AQFL	Aqueous soluble fraction of leaf
Stem Bark	MEB	Methanolic soluble fraction of stem bark
	PEFB	Petroleum soluble fraction of stem bark
	CTFB	Carbon tetrachloride soluble fraction of stem bark
	CHFB	Chloroform soluble fraction of stem bark
	AQFB	Aqueous soluble fraction of stem bark

2.2. In Vitro Experiments

2.2.1. Total Phenolic Content

Gallic acid was employed as the standard in the Folin-Ciocalteau's spectrophotometric method to calculate the total phenolic content [12]. Gallic acid solutions in a range of concentrations from 0.391 μg/mL to 100 μg/mL were prepared. Next, 2.5 mL of a 10-fold diluted Folin-Ciocalteau's phenol reagent was mixed with 0.5 mL of the plant extract (2 mg/mL). Subsequently, 2.0 mL of a 7.5% sodium carbonate (Na_2CO_3) solution was added, and the mixture was left to stand in the dark for 20 min. Finally, the absorbance was measured at 760 nm and the total phenolic content of the plant extracts was determined using a calibration curve.

2.2.2. DPPH Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured using the Brand-Williams technique [13]. As reference standards, tert-Butyl-1-hydroxytoluene (BHT) and ascorbic acid (AA) were employed. The following formula was used to determine the percentage (%) of DPPH scavenging activity:

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

where, A = Absorbance of each group.

2.2.3. Membrane-Stabilizing Activity

Hypotonic solution- and heat-induced hemolysis experiments were performed to assess the membrane-stabilizing activity of the plant extracts [14].

2.2.4. Anthelmintic Activity

Pheretima posthuma (Annelida) was utilized to measure the in vitro anthelmintic activity of each *B. malabarica* extract at three different concentrations (20, 10, and 5 mg/mL), with albendazole and saline water serving as the standard and control, respectively.

2.2.5. Brine Shrimp Lethality Bioassay

In this experiment, brine shrimp eggs were hatched into nauplii by incubating them in seawater simulation [15]. To achieve the required concentration of the test sample, a precisely measured quantity of dimethyl sulfoxide (DMSO) was added. The nauplii were counted, placed in vials containing 5 milliliters of artificial saltwater. A micropipette was employed to add different concentrations of the samples to the vials that had already been labeled. The number of surviving nauplii was noted after a 24-h period.

2.3. In Vivo Experiments

2.3.1. Test Animals and Study Design

For the study, Swiss-albino mice of either sex, 4 to 5 weeks of age, were acquired from the pharmacy department of Jahangirnagar University in Savar, Dhaka, Bangladesh. Using a 12-h light/dark cycle for adequate ventilation, the mice were kept in standard polypropylene cages with controlled temperatures (24 ± 2 °C, 60–70% relative humidity). They were fed a regular diet, commercially available from vendors, with water provided ad libitum. The animals were allowed to acclimatize to the laboratory settings for 1 week and then divided into different experimental groups randomly. Twelve hours before and throughout the trial, the mice were fasted. All animal procedures were approved by the institutional ethical committee [16].

As part of the study design, eighteen experimental animals were chosen at random and divided into six groups (Group I, Group II, Group III, Group IV, Group V, and Group VI). Each group consisted of three mice and received a different therapy. The dosages of the test items and control materials were adjusted appropriately, and each mouse was carefully weighed before the experiment began. Animals in a group were given distinct identities in order to let them be identified during the therapy (Numbering of Mice): Mice 1 = M1; Mice 2 = M2; and Mice 3 = M3.

2.3.2. Central Analgesic Activity

The tail immersion test [17] was used to assess the central analgesic effect of the plant extracts. Morphine (2 mg/kg body weight) solution was administered subcutaneously as the standard. The *B. malabarica* extracts and the negative control (1% Tween 80 in normal saline) were administered orally at a dosage of 200 and 400 mg/kg body weight. The percentage (%) of time elongation relative to the control mice was determined using the following formula:

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

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

2.3.3. Peripheral Analgesic Activity

Acetic acid-induced writhing was utilized to test the plant extract's peripheral analgesic effect in albino mice [18]. Albino mice were administered the plant extracts at 200 and 400 mg/kg body weight, as well as the conventional reference medication, diclofenac sodium (50 mg/kg body weight), and 1% Tween 80 in normal saline as the negative control. About 40 min after the sample was delivered, the experimental animal was given 0.1 mL/10 g body weight of 1% acetic acid intraperitoneally to induce writhing.
The following formula was used to determine the percentage of writhing inhibition:

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

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

2.3.4. Anti-diarrheal Activity

The castor oil-induced diarrhea model in mice was used to evaluate the anti-diarrheal properties of *B. malabarica* [19]. The test groups received 200 mg and 400 mg/kg of body weight of the soluble fractions of the crude methanol extract of *B. malabarica*. The negative control group was administered a vehicle solution (10 mL/kg body weight) that contained 1% Tween 80 in normal saline, while the positive control group was administered 50 mg/kg body weight of loperamide orally. The number of diarrheal stools produced by the mice was monitored for a maximum of four hours, and the percentage reduction in diarrhea caused by the plant extract was noted.

The percentages (%) of diarrhea decrease were calculated using the following formula:

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

D = The number of cases of diarrhea in each group.

2.3.5. Sedative Activity

The phenobarbitone-induced sleeping time test was used to assess the sedative effects of *B. malabarica* leaf and bark extracts on Swiss albino mice [20]. The test groups were administered 200 mg/kg and 400 mg/kg of the leaf and bark extract orally. Diazepam (1 mg/kg, i.p.) served as the traditional positive control in this investigation. Phenobarbitone sodium (25 mg/kg body weight) was given intraperitoneally to induce sleep after a 30-min break. The onset time (in min) and the duration of sleep (from the loss to the recovery of the righting reflex) were recorded as indicators of sedative activity [21].

3. Results

The purpose of this study was to evaluate cytotoxic, antioxidant, membrane-stabilizing, anthelmintic, analgesic, anti-diarrheal, and sedative properties of *B. malabarica*.

3.1. Total Phenolic Content

A comparative analysis of the total phenol content (TPC) of all *B. malabarica* leaf extract and stem bark extracts along with various solvent fractions was carried out using the spectrophotometric method. Using the calibration curve obtained from gallic acid solutions and the regression equation ($y = 0.016x + 0.021$, $R^2 = 0.998$), the total phenol concentration in the extracts was ascertained. Table 2 shows that the total phenolic content (TPC) of *B. malabarica* leaf extracts ranged from 1.51 to 73.49 mg of GAE per gram. At 73.49 mg of GAE/g, the methanol leaf extract (MEL) had the greatest phenolic content. The petroleum ether leaf extract (PEFL) and chloroform leaf extract (CTFL) had TPC values of 28.06 and 32.38 mg of GAE/g, respectively. Out of all the extractives used for *B. malabarica* stem bark, CHFB had the highest phenolic content (10.28 mg of GAE/gm of extractives), followed by MEB (7.13 mg of GAE/gm of extractives).

This could be because these two fractions contain a considerable amount of antiradical phenolic compounds.

Table 2. Crude methanol extracts and their fractions from *B. malabarica* leaves and bark are examined for their cytotoxic effects, antioxidant qualities, and total phenolic content.

Test Sample	Antioxidant Activity		Cytotoxic Activity
	Total Phenolic Content (mg of GAE/g of Dried Extract)	DPPH Assay IC_{50} (μg/mL)	LC_{50} (μg/mL)
MEL	73.49	12.15	1.16
PEFL	28.06	45.32	2.81
CTFL	32.38	35.60	1.73
CHFL	6.57	22.81	3.52
AQFL	1.51	29.45	1.45
MEB	7.13	20.95	4.23
PEFB	2.44	38.20	28.09
CTFB	4.10	31.88	4.23
CHFB	10.28	18.22	8.97
AQFB	3.06	25.75	2.09
BHT	-	22.50	-
AA	-	5.80	-
VS	-	-	0.45

3.2. DPPH Free Radical Scavenging Activity

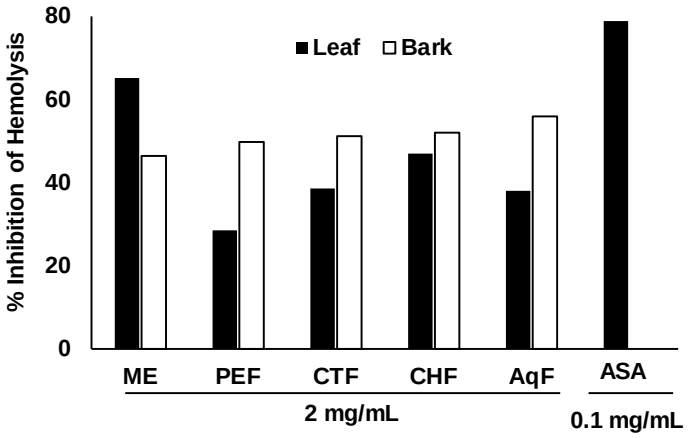
The ability of the organic components of the *B. malabarica* extract to scavenge DPPH free radicals was used to measure its antioxidant activity. With an IC_{50} value of 12.15 μg/mL, the methanolic leaf extract showed the highest free radical scavenging activity, as shown in Table 2. The chloroform fraction of the stem bark followed in next with an IC_{50} of 18.22 μg/mL. The results of the study showed that the improved antioxidant activity of the test sample is roughly close to that of conventional ascorbic acid (IC_{50} = 5.8 μg/mL). This could be because these two fractions contain a considerable amount of antiradical phenolic compounds (Table 2). This assay is based on the scavenging of DPPH free radicals [22] by antioxidants due to their hydrogen-donating capability [23].

3.3. Brine Shrimp Lethality Bioassay

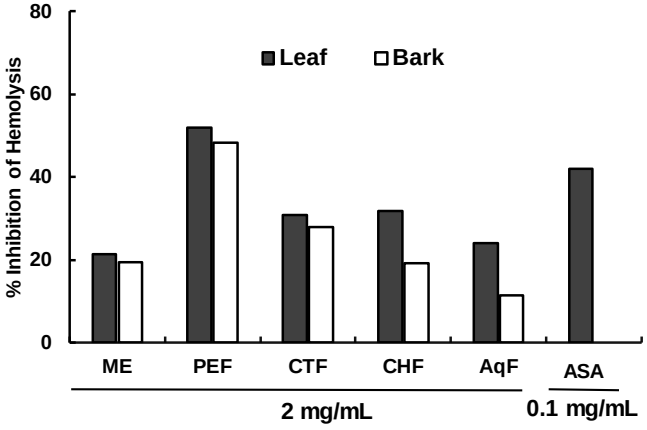
For the initial evaluation of plant extract cytotoxicity, the brine shrimp lethality test is frequently utilized. Cytotoxic compounds in this assay show significant activity, making it a useful, low-cost method for detecting anti-tumor and pesticidal compounds [24]. Table 2 presents the LC_{50} values for the plant extracts and the positive control, vincristine sulphate. All *B. malabarica* extracts displayed notable toxicity to brine shrimps, with LC_{50} values ranging from 1.16 to 28.09 μg/mL, compared to vincristine sulphate's LC_{50} of 0.45 μg/mL, suggesting the extracts as potential candidates for anticancer compounds. The methanol extract of leaf and its carbon tetrachloride and aqueous fraction showed more cytotoxic effects with LC_{50} value of 1.16, 1.73 and 1.45 μg/mL, respectively. Similarly, *B. malabarica* leaf methanol extract's aqueous fraction showed notable cytotoxicity (LC_{50} = 2.09 μg/mL) against shrimp nauplii (Table 2). Higher extract concentrations gradually boosted the plant sample's inhibitory impact. This suggests the possibility that the extract contains harmful substances, which warrants further investigation.

3.4. Membrane-Stabilizing Activity

The various soluble fractions from the methanol extract of *B. malabarica* were tested for membrane stabilizing activity using standard protocols, with the results presented in Figure 1. At a concentration of 2.0 mg/mL, the plant samples significantly reduced RBC hemolysis induced by both hypotonic solution and heat, outperforming the standard aspirin (Figure 1A, 1B). In the hypotonic solution-induced hemolysis model, the methanol extracts of both the leaf and bark showed 65.2% and 46.4% inhibition of RBC hemolysis, respectively, compared to the reference aspirin, which inhibited 78.0% of hemolysis (Figure 1A). During this experiment, most of the solvent fractions notably the pet-ether fraction (49.9%), carbon tetrachloride fraction (51.2%) and aqueous fraction (56.0%) of the *B. malabarica* bark showed prominent inhibitory activity against the hemolysis induced by hypotonic solution. In case of heat induced hemolysis, all these solvent fractions from the methanol extract of *B. malabarica* leaf showed stronger protection activity compared to the extent of inhibition observed by the solvent fractions of *B. malabarica* bark (Figure 1B).



(**A**)



(**B**)

Figure 1. The RBC membrane stabilizing properties of different organic fractions of *B. malabarica* leaf and bark extract were determined using the hypotonic solution-induced hemolytic method **(A)** and Heat-induced method (**B**).

3.5. Anthelmintic Activity

In comparison to the negative control, the methanol extract of leaves and bark of *B. malabarica* considerably shortened the paralysis and death times of *Pheretima posthuma* (Table 3). This anthelmintic activity was dose-dependent and compared to the standard albendazole at the concentration tested. Here the methanol extract of leaf, MEL at the concentration of 20, 10 and 5 mg/mL showed paralyzing time 29, 36, 48 min and death time 62, 75, 83 min, respectively. Similarly, normal albendazole (Conc. 10 mg/mL) induced paralysis at 25 min and death at 55 min, whereas the methanol extract of *B. malabarica* bark was most efficacious at a concentration of 20 mg/mL, causing paralysis and death within 31 min and 67 min, respectively.

Table 3. Anthelmintic effects of the methanol extracts from the leaves and stem bark of *B. malabarica* where n = 1.

Test Sample	Concentration (mg/mL)	Paralyzing Time (min)	Death Time (min)
Albendazole	10	25	55
MEL	20	29	62
	10	36	75
	5	48	83
MEB	20	31	67
	10	39	79
	5	50	97

3.6. Central Analgesic Activity

The central analgesic effects of standard morphine and organic soluble fractions in albino mice, as determined by the tail immersion method, are summarized in Table 4. A higher percentage of elongation indicates stronger central antinociceptive activity. The results revealed that all plant extracts at doses of 200 and 400 mg/kg body weight significantly ($p < 0.001$) and dose-dependently reduced pain sensation compared to the untreated control groups. However, morphine (2 mg/kg body weight) showed a higher analgesic effect than the plant extracts ($p < 0.001$). At 30, 60, and 90 min after the albino mice were given the plant samples, the percentage elongation times were noted. Mice given 200 and 400 mg/kg body weight dosages of the methanolic leaf extract showed substantial tail immersion elongation of 55.63 ± 0.587%, 144.30 ± 0.760%, and 167.57 ± 0.309% at 30, 60, and 90 min, respectively. In comparison, standard morphine resulted in an elongation of 447.63%. Likewise, the maximum analgesic effect of the methanolic bark extract was observed at 90 min, with a 209.34 ± 0.163% increase in reaction time after treatment with 400 mg/kg in mice (Table 4). These findings show that, in comparison to the morphine solution, the methanolic leaf and bark extracts both considerably ($p < 0.001$) postponed the start of pain sensation.

Table 4. *B. malabarica* leaf and bark extract's central analgesic effects in mice.

Test Sample	% Time Elongation (Mean ± SEM)		
	After 30 min	After 60 min	After 90 min
Morphine 2 mg/kg	106.11 ± 0.492 ***	267.15 ± 0.780 ***	447.63 ± 0.409 ***
MEL 200 mg/kg	55.63 ± 0.587 *	144.30 ± 0.760 **	167.57 ± 0.309 ***
MEL 400 mg/kg	73.20 ± 0.638 **	172.40 ± 1.060 **	193.94 ± 1.306 *
MEB 200 mg/kg	35.57 ± 0.912	130.94 ± 0.373 ***	143.57 ± 0.193 ***
MEB 400 mg/kg	41.98 ± 0.580 *	187.79 ± 0.719 **	209.34 ± 0.163 ***

Values are presented as Mean ± (standard error mean) SEM and (n=3); * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ were considered as significant.

3.7. Peripheral Analgesic Activity

Figure 2 depicts the effects of methanolic leaf and bark extracts of *B. malabarica* on acetic acid-induced abdominal writhing in mice. The findings show that, in comparison to the negative control group, the usual medication, diclofenac sodium (2 mg/kg body weight), and the plant extracts (at 200 and 400 mg/kg body weight) considerably ($p < 0.01$) decreased abdominal writhing. The methanolic leaf extract exhibited gradual inhibition from 0% in the control group to 58.32 ± 1.00% at 200 mg/kg and 69.51 ± 0.58% at 400 mg/kg body weight. There was a dose-dependent decrease in writhing. Similar to the standard drug diclofenac (which showed 76.73 ± 0.58% inhibition of the writhing response), the bark extract of *B. malabarica* exhibited dose-dependent anti-nociceptive effects, with 44.9 ± 0.88% and 53.06 ± 0.33% suppression of writhing at doses of 200 and 400 mg/kg body weight, respectively, in mice (Figure 2).

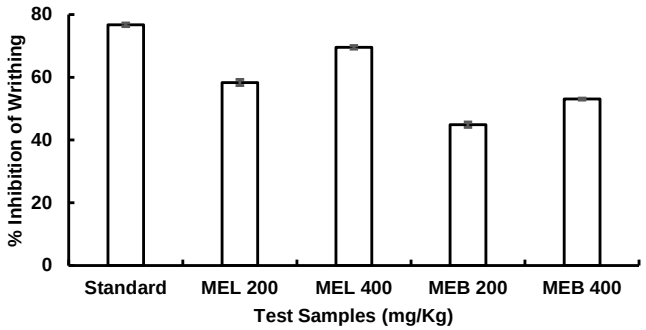


Figure 2. Response of the methanol extract of *B. malabarica* leaves and bark to peripheral analgesia. All values are represented as Mean ± SEM (standard error mean), (n = 3).

3.8. Anti-Diarrheal Activity

The leaf and bark extracts, at 200 and 400 mg/kg body weight, significantly ($p < 0.05$) decreased the overall number of diarrheal stools in the castor oil-induced diarrheal model. The leaf extract inhibited diarrhea by 42.31 ± 2.11% at 200 mg/kg and 59.23 ± 1.78% at 400 mg/kg, while the bark extract showed 53.54 ± 1.38% and 61.54 ± 1.49% inhibition at the same doses, respectively. In comparison, the standard drug loperamide achieved a 69.23 ± 1.49% inhibition (Figure 3).

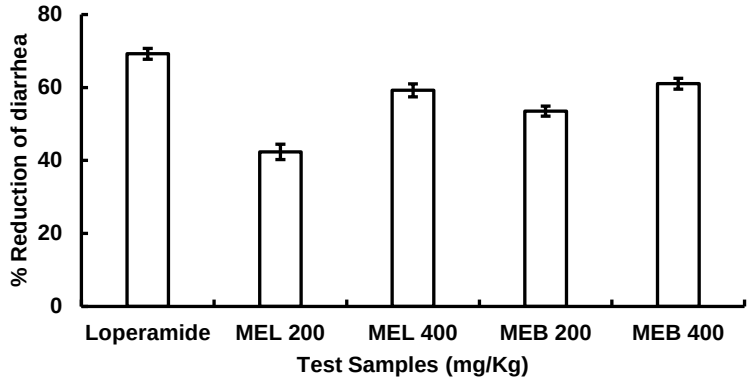


Figure 3. In the anti-diarrheal activity test, oral administration of different doses (200 and 400 mg/kg body weight) of the methanol extracts from the leaf and bark of *B. malabarica* (MEB) significantly ($p < 0.05$) reduced the number of diarrheal feces in mice (n = 3). These results were more pronounced at higher extract dosages.

3.9. CNS Antidepressant Activity

The phenobarbitone sodium-induced sleeping time test was conducted to evaluate the sedative effects of *B. malabarica*. In a dose-dependent manner, the leaf and bark extracts decreased the onset of sleep and increased its length, similar to the control group (Figure 4). The overall sleep lengths were roughly 89.7 and 99 min when the leaf extract was given at doses of 200 and 400 mg/kg body weight. The sleep start timings were 62.33 and 58.0 min, respectively. On the other hand, total sleep durations were 117.3 and 128.3 min, respectively, when the bark extract was administered at 200 and 400 mg/kg (Figure 4). These findings imply that extracts decreased locomotor activity, as demonstrated by the mice's longer sleep durations. The sedative effects may be attributed to interactions with benzodiazepine-like compounds, potentially enhancing GABAergic inhibition in the CNS [25].

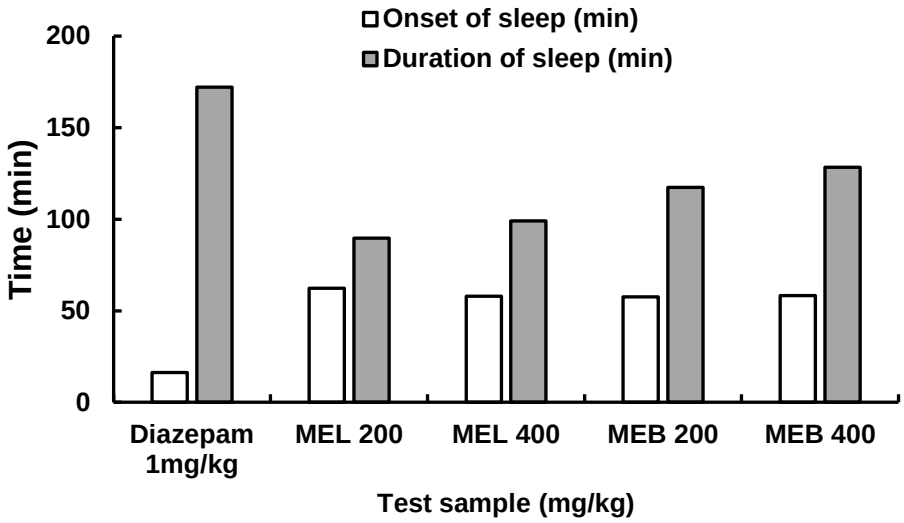


Figure 4. Sedative effect of leaf and bark extract of *B. malabarica* (n = 3).

4. Discussion

In this study, we explored the bioactive potential of *B. malabarica* through a series of in vitro and in vivo assays to assess its antioxidant, anti-inflammatory, analgesic, anti-diarrheal, anthelmintic, and sedative properties. The results of these experiments demonstrated that *B. malabarica* exhibits a range of bioactivities, making it an effective traditional medicine.

The DPPH free radical scavenging method was employed to evaluate the antioxidant activity of *B. malabarica*. It is well-known that free radicals play a key role in the pathogenesis of various diseases, including diabetes, atherosclerosis, and aging. Antioxidants counteract free radical damage, protecting cellular biomolecules like lipids, proteins, and nucleic acids. Our results demonstrated that the methanolic extract of *B. malabarica* leaves exhibited significant free radical scavenging activity, suggesting its potential as an effective antioxidant

(Table 2). These findings confirm that the plant can combat oxidative stress, which participates in diverse chronic diseases [26,27].

In addition to its antioxidant effects, *B. malabarica* also showed promising antitumorigenic potential. The methanolic leaf extract exhibited better results compared to vincristine sulfate, a known chemotherapy agent, in the current bioactivity studies. This suggests that the plant may possess components capable of modulating tumorigenic processes. Many phytochemical sources can promote the development of antitumor agents by inducing apoptosis in cancer cells [28], further supporting the potential therapeutic applications of *B. malabarica* in cancer prevention or treatment.

The RBC membrane stabilization test assessed the anti-inflammatory potential of the plant by evaluating its ability to protect red blood cell membranes from hemolysis induced by heat or hypotonic solutions [29]. Phytochemicals such as flavonoids, tannins, and other phenolic compounds, present in both the leaf and bark extracts of *B. malabarica*, likely contribute to the membrane-stabilizing activity observed in these assays. The leaf extract exhibited stronger protective effects compared to the bark, as shown in Figure 1B. This suggests that the anti-inflammatory properties of the plant may be due to its ability to stabilize cellular membranes and prevent damage from inflammatory stressors.

To determine the plant's dose-dependent anthelmintic activity, we evaluated paralysis and death times of worms exposed to the extracts. These results support the traditional use of *B. malabarica* as an anthelmintic agent. These laboratory findings reinforce the need for alternative anthelmintic treatments, especially in regions where parasites are developing resistance to synthetic drugs [30,31]. The ability of the plant to rapidly induce paralysis and death in helminths highlights its potential for further development into an affordable and effective anthelmintic therapy.

The analgesic effects of *B. malabarica* were evaluated using both the tail immersion test (for central analgesic effects) and the acetic acid-induced writhing test (for peripheral analgesic effects). Both tests demonstrated that the soluble fractions from the leaf and stem bark extracts significantly reduced pain in Swiss albino mice at doses of 200 and 400 mg/kg. These findings suggest *that B. malabarica* may possess both central and peripheral analgesic properties. The extracts may modulate pain via interacting with the central nervous system and peripheral chemoreceptors, as evidenced by their ability to reduce abdominal constrictions induced by acetic acid [32–35].

The anti-diarrheal effects of *B. malabarica* were examined in mice treated with castor oil, which induces diarrhea by irritating the gut mucosa. The plant's extracts significantly reduced the incidence of diarrhea, indicating that it may be effective in managing gastrointestinal disorders. The presence of bioactive compounds such as tannins, terpenes, alkaloids, glycosides, and flavonoids likely contribute to this effect, as they are known to have astringent, anti-inflammatory, and gut-modulating properties [36].

The phenobarbitone sodium-induced sleeping time test was conducted to evaluate the sedative properties of *B. malabarica*. The leaf and bark extracts exhibited a dose-dependent effect, decreasing the onset of sleep and prolonging the duration of sleep in mice. The leaf extract at doses of 200 and 400 mg/kg body weight resulted in sleep durations of 89.7 and 99 min, respectively, while the bark extract caused sleep durations of 117.3 and 128.3 min (Figure 4). These results suggest that *B. malabarica* may possess sedative properties, possibly through interactions with the GABAergic system in the central nervous system [37]. The plant's ability to reduce locomotor activity and prolong sleep could make it a potential candidate for further investigation as a sedative or anxiolytic agent.

5. Conclusions

In conclusion, this study highlights the diverse bioactive properties of *B. malabarica*, supporting its traditional use in herbal medicine. The extracts showed notable sedative, anti-diarrheal, anthelmintic, cytotoxic, antioxidant, and membrane-stabilizing properties. Based on the biological activities, *B. malabarica* could be a valuable resource for developing therapeutic agents. These findings need to be further investigated to fully elucidate the mechanisms underlying these effects and to explore the potential applications of *B. malabarica* in modern medicine.

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