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ACUTE TOXICITY AND PHARMACOLOGICAL EFFECTS OF METHANOLIC LEAVE EXTRACT OF *ORMOSIA ROBUSTA* (FABACEAE)

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ABSTRACT

Ethnopharmacological relevance: Ormosia robusta (fabaceae) is a perennial non-climbing woody tree.

***Objective:** The current study sought to investigate the phytochemical screening, antioxidant properties, membrane stabilizing activity, anticonvulsant activity, and gastrointestinal motility properties of O. robusta methanolic leaf extract.*

***Materials and methods:** Phytochemical analysis was carried out using standard methods, and antioxidant activity was determined using the estimation of total phenolic contents and DPPH radical scavenging activity. Hypotonic solution-induced haemolysis and Heat-induced haemolysis tests were used to investigate membrane stabilizing activity. The anticonvulsant activity was evaluated using Isoniazid (INH) induced convulsions and Pilocarpine induced convulsions methods in mice. And gastrointestinal motility test was carried out by the charcoal meal passage test in mice using methanolic leaf extract of O. robusta.*

***Results:** Preliminary phytochemical examination showed the presence of phytosterols, phenol, glycosides, tannins, terpenoids, flavonoids, saponin, protein and amino acid. The methanolic leaves extract of O. robusta (200 and 400 mg/kg; i.p.) produced significant ($p < 0.01$) dose-dependent inhibition of the latency of convulsion responses elicited by Pilocarpine and Isoniazid. The membrane-stabilizing action of the extract was demonstrated by its ability to prevent hypotonic and heat-induced haemolysis. Methanolic extract showed significant antioxidant properties. In respect of the gastrointestinal motility test, O. robusta caused significant ($p < 0.001$) dose-dependent inhibition of motility in the mice. The effects of the extract in the various models were generally comparable to those of the standard drugs used.*

***Conclusion:** The findings in this study suggest that the methanolic leaves extract of O. robusta possesses antioxidant, membrane stabilizing activity, gastrointestinal motility properties and anticonvulsant effect possibly mediated through several mechanisms. These activities could be attributed to the presence of tannins, terpenoids, phenols, saponins and flavonoids.*

KEYWORDS: *Ormosia robusta baker, antioxidant properties, membrane stabilizing activity, anticonvulsant activities, gastrointestinal motility*

INTRODUCTION

The investigated plant *O. robusta* baker belongs to the family Fabaceae (also known as the legume family: Leguminosae), locally known as Sanchi or Sanchi-blog by the tribe garos (Sinha et al. 2014). It was found growing on the hilly terrain of tropical semi-evergreen forests. It is widely distributed throughout East Asia, some parts of India (Assam, Arunachal Pradesh, Meghalaya, Mizoram), Myanmar, and Thailand. In Bangladesh, it is found in the hills of sitakunda and maheskhali (Misbahuzzaman, K., Alam 2006). *O. robusta baker* is a large tree, up to 12 m, with bright orange-colored bunches of fruits. Leaves are imparipinnate, flowers creamy-white, legumes 1-2 seeded, cylindric or slightly compressed between seeds looked yellowish-green. It is mainly used as timber and firewood. The extract of bark (soaked in water overnight) is used in the treatment of jaundice by the garos (Ghani 2002; Sinha et al. 2014). Although this plant is not widely explored yet, the bark has been reported to contain two prenylated isoflavones, warangalone and erysenegalensein m, along with two triterpenoids, betulinic acid and lupeol (Ahmed, Haque, and Ahsan 2013). Based on the literature search, no study has been carried out to scientifically use *O. robusta* in treating RBC haemolysis, diarrhea and convulsive disorders. The present study aimed to find the phytochemical constituents, acute toxicity, antioxidant and membrane stabilizing activity, anticonvulsant activities and gastrointestinal motility test of the methanolic leaves extract of *O. robusta Baker.*

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2. MATERIAL AND METHODS

Plant Collection and Extract Preparation

The leaves of *O. robusta Baker* (Fabaceae) were collected from Maheskhali Island, Chittagong, Bangladesh. The plant was identified at Bangladesh National Herbarium, Dhaka, Bangladesh, and a voucher specimen (Accession no. DACB 38324) was deposited for future reference. The collected plant parts were separated from undesirable materials or plants or plant parts and washed thoroughly with water several times. During Collection, any adulteration was strictly prohibited. They were sun-dried for one week. The leaves were grounded into a coarse powder with the help of a suitable grinder. Dried powdered samples (500 gm) were immersed in 3000 ml of 90% methanol (Merck KGaA, Darmstadt, Germany) with intermittent shaking. Following 15 days, the solvent was decanted, filtered, and evaporated in a rotary evaporator. The extract was kept protected from light and stored at 4◦C (yield 15 g deep greenish gummy extract. The Greenish gummy extract obtained was always reconstituted in distilled water to the appropriate concentration before administration to experimental animals.

Laboratory Animals

Swiss-albino mice of either sex (aged 4-5 weeks, weighing 18-25gm) obtained from the Laboratory mice-breeding centre, the department of pharmacy, Jahangirnagar University, were used for the studies. The animals were housed in well-ventilated cages that were kept under normal environmental circumstances and had free access to food and water. Mice experiments were conducted in compliance with the regulations of the Animal Ethical Committee of the Noakhali Science and Technology University Research Cell. These guidelines followed the internationally accepted principles for laboratory use and care.

Drugs, Chemicals and Reagents

All of the chemicals utilized were of analytical reagent quality. Methanol was purchased from Merck, Darmstadt, Germany; Castor oil was purchased from WELL's Heath Care, Spain. Pilocarpine hydrochloride **(**Popular Pharmaceuticals Ltd. Bangladesh), Isoniazid (Novartis (Bangladesh) Ltd.), Hyoscine butyl bromide (ACI Ltd. Bangladesh), Activated Charcoal (Lab. Reagent, India), DPPH (1,1-diphenyl, 2-picryl hydrazyl), trichloroacetic acid, ferric chloride, Gallic acid and BHA were obtained from Sigma Chemical Co. USA. Folin-Ciocalteu reagent, sodium carbonate and potassium ferricyanide were purchased from Merck, Germany. Hydrochloric Acid (BDH Ltd, England), Chloroform (ACS, Merck), Ammonia (Merck Millipore, India), Ferric Chloride (Fisher, USA). Acetic anhydride, Sulphuric acid, lead acetate, Nitric acid, and Copper acetate were also purchased from Merck, Darmstadt, Germany. All other reagents were procured from Sigma Chemicals limited.

Phytochemical Screening

A preliminary phytochemical study was screened for the presence of alkaloids, phenols, phytosterols, Saponins, proteins and amino acids, flavonoids, and terpenoids. These were identified by characteristic colour changes using standard procedures (J.B. Harborne 1998).

Acute toxicity test

Five groups of 3 mice, each fasted for 18 hr prior to the experiment, were administered. A study of acute toxicity was conducted using OECD guideline 423. ORE (*O. robusta* extract) was administered orally to Swiss albino mice (20-25mg, n=3) at doses of 1000, 1500 and 2000 mg/kg p.o. Animals in the different groups were observed for 2 hr post-treatment for immediate signs of toxicity and behavioural changes (abdominal constriction, hyperactivity, sedation, grooming). Mortality and body weight were observed for 72 hrs.

Antioxidant test

Estimation of total phenolic content

The phenolic content of plant extracts was measured using the Folin-Ciocalteu reagent. Due to the use of gallic acid as a standard, the total phenolic content was reported as mg/g of gallic acid equivalents (GAE). The concentration of 6.25, 12.5, 25, 50, and 100 mg/ml of gallic acid. Stock solution (0.5 mg/ml) of plant extracts was prepared and diluted to five different concentrations (0.3, 0.2 and 0.1 mg/ml). Then 0.5 ml of sample was introduced into test tubes, and 2.5 ml of a 10-fold dilute Folin-Ciocalteu reagent (Sigma–Aldrich) was added to the extract concentrations in different sets of test tubes, shaken thoroughly, and left to stand for one min. 2 ml of 7.5% $NaHCO_3$ was then added and the mixture once again allowed to stand for 30 min at room temperature. The absorbance of the supernatant was measured at 760 nm using UV spectrophotometry (UV-1800, Shimadzu, Japan). The total phenolic content was calculated as mg of gallic acid equivalent per gram using a standard gallic acid calibration curve (Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal 2006).

Free radical scavenging activity by DPPH method

the free radical scavenging activity of *leaf* extract was evaluated using the stable radical DPPH (1,1-diphenyl, 2-picryl hydrazyl) according to the method described by Hazra et al. (Hazra, Biswas, and Mandal 2008). Plant methanol extracts (2 ml) were mixed with DPPH (1 ml, 0.5 mM) and sodium acetate buffer (pH 5.5, 2 ml, 0.1 M) and incubated for 30 min at room temperature. The supernatant absorbance was measured at 517 nm using a UV spectrophotometer against a blank of methanol. The lower absorbance of the reaction mixture was indicative of increased free radical scavenging activity. The capacity to scavenge the DPPH radical was computed using the following equation:

$$\%\ \text{radical scavenging activity} = \frac{\text{A control} - \text{A test sample}}{\text{A control}} \times 100$$

Here, A represents absorbance.

The inhibition percentage versus extract/standard concentration was displayed on a graph, and the extract concentration resulting in 50% inhibition (IC_{50}) was computed.

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Membrane stabilizing activity

The erythrocyte membrane is comparable to the lysosomal membrane; therefore, the effect of medications on the stabilization of the erythrocyte membrane could be extended to the stabilization of the lysosomal membrane. The membrane stabilizing activity of the extractives was assessed by using hypotonic solution-induced and heat-induced erythrocyte haemolysis of human erythrocytes by the method developed by Omale and Okafor (Omale and Okafor 2008) and Shinde *et al*. (Shinde, U.A., Phadke, A.S., Nair, A.M., Mungantiwar, A.A., Dikshit, V.J. and Saraf et al. 1999).

Collection of blood samples

In the current study, 2 ml of blood was drawn from each of the healthy Bangladeshi male humans (70kg) volunteers (n=5, age 20 to 23 years) who had no history of oral contraceptive or anticoagulant therapy. The obtained RBCs were stored in test tubes with anticoagulant Ethylene diamine-tetraacetic acid (EDTA) at room temperature (232°C). The supernatant absorbance was measured at 760 nm by UV spectrophotometry (UV-1800, Shimadzu, Japan). Using the equation derived from a standard gallic acid calibration curve, the total phenolic content was calculated as mg of gallic acid equivalent per gram (Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal 2006).

Preparation of erythrocyte suspension

To prepare the stock erythrocyte (RBC) suspension, 2 ml of blood was drawn from male volunteers' anti-cubital veins. The blood sample was centrifuged at 3000xg for 10 minutes and then rinsed three times with isotonic solution (0.9% saline). The volume of saline was determined and reconstituted as a 40% (v/v) suspension with an isotonic buffer solution (pH 7.4) containing 1 L of distilled water: NaH2PO4. 2H2O, 0.26 g; Na2HPO4. 1.15 g; NaCl, 9 g. (10 mM sodium phosphate buffer).

Hypotonic solution-induced haemolysis

0.50 ml of stock erythrocyte (RBC) suspension was combined with 5 ml of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate-buffered saline (pH 7.4) containing either extract (1.0 mg/ml) or acetylsalicylic acid (0.1 mg/ml) for the test sample. The control sample, including 0.5 ml of RBCs, was combined with only hypotonic-buffered saline. Following 10 minutes of incubation at room temperature, the mixture was centrifuged at 3000xg for 10 minutes, and collect the supernatant. Supernatant absorbance was measured at 540 nm using an ultraviolet spectrophotometer. The % inhibition of either haemolysis or membrane stabilization was computed using the following equation.

$$\%\ \text{inhibition of haemolysis} = \frac{OD1 - OD2}{OD1} \times 100$$

OD1 = Optical density of hypotonic-buffered saline solution alone (control) and

OD2 = Optical density of test sample in a hypotonic solution

Heat-induced haemolysis

1.0 mg/ml of plant extract or vehicle was dissolved in 5 ml of the isotonic buffer (pH 7.4), and 30 µl of erythrocyte suspension was added. Following 20 minutes of incubation at 54°C in a water bath, the tubes were cooled to 5°C in an ice bath and centrifuged at 1300xg for 3 minutes. The supernatant absorbance was determined at 540 nm using a UV spectrometer. Each sample set was evaluated in duplicate. The percentage inhibition or acceleration of haemolysis was calculated using the following equation:

$$\%\ \text{inhibition of haemolysis} = \frac{OD1 - OD3}{OD1} \times 100$$

Where, OD1 = test sample unheated; OD2 = test sample heated and OD3 = control sample heated

Anticonvulsant activities

Mice were observed continuously for 30 minutes following pilocarpine and INH administration for the occurrence of limbic seizures and status epilepticus. The latency to the first episode of convulsive behavior (forelimb clonus), duration of convulsion and percentage mortality were recorded for a period of 30 min. Animals surviving more than 30 min were considered to be protected.

Maximal Electrical Shock (MES) induced seizures:

Four groups of six Swiss albino mice (25-30 g) of either sex were used. Seizures are usually induced in mice by delivering electroshock (50 mA for 0.2 seconds) by means of an electro-convulsio meter through a pair of corneal electrodes. The test animals received 200 and 400 mg/kg of ORE orally, and the standard group received phenytoin (25 mg/kg body weight) injected intraperitoneally. Thirty minutes later, MES-induced seizure was recorded, and protections from HLTE were recorded k and protections from were recorded response. All the experimental groups were compared with the control treated with a vehicle. The disappearance of the tonic hind limb extensor was used as a positive criterion. The percentage of inhibition of seizures relative to control was calculated.

Isoniazid (INH) Induced Convulsions

Albino mice (18-22 g) of either sex were randomly divided into four groups (n=5) and fasted overnight before the experiment; however, the water was supplied ad libitum. Group I was maintained as control and was given INH (300 mg/kg i.p.) only, Group II- Standard received Diazepam (5mg/kg i.p.), Group III-methanolic leaves extract (200mg/kg i.p.) and Group IV-methanolic leaves extract (400mg/kg i.p.). Group III and IV were administered extracts into mice at doses of 200 and 400 mg/kg 1h before i.p. administration of INH (300 mg/kg, i.p.) whereas, in Group II, INH was injected after 30 min of diazepam (5 mg/kg, i.p) treatment. The mice were placed in an isolated perplex chamber and observed for the following 30 minutes for the onset and duration of convulsion, which include clonic seizures, the extension of the hind limb, Fictive scratching, Tremors, Stupor and percent protection. The

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percentage of seizures or deaths in the control group was 100%. The suppression of these effects in the treated groups was calculated as a percentage of controls (Bum et al. 2010; Rang HP, Dale MM, Ritter JM 2003).

Pilocarpine Induced Convulsions

Pilocarpine-induced convulsions were done with the method of Patrick *et al.*(Doughari et al. 2012) and Luciana *et al*. (Luciana et al. 2012) with slight modification. In this experiment, seizures were induced by an i.p. injection of Pilocarpine (240 mg/kg) into drug or vehicle-treated male mice. Albino mice (18-22 g) were divided into four groups (n=5 mice of either sex in one group). Group I received respective vehicles (10ml/kg); Group II was allotted for standard drug (Diazepam 5mg/kg i.p.), and Group III and IV received ME (200 mg/kg and 400 mg/kg i.p.) at different doses levels respectively. After 1 hr of injecting distilled water, Diazepam and ME into mice, Hyoscine butyl bromide (1 mg/kg i.p.) was administered. The animals were pretreated with Hyoscine butyl bromide to minimize the peripheral autonomic effects of Pilocarpine. Pilocarpine was administered to mice of Group -II at 15 minutes and group- I, III and IV at 30 minutes, respectively, after Hyoscine butyl bromide injection. After the injection of the Pilocarpine, the animals were placed separately into the transparent Plexiglas testing chamber and recorded data as previously described in the INH experiments.

Gastrointestinal motility test

Experimental procedure described by Dosso *et al*. (Dosso K., N'guessan, B.B., Bidie, A.P., Gnangoran, B.N., Méité, S., N'guessan, D. 2011). For this purpose, the mice of either sex (25 to 35 g) fasted 18 to 24 hr before starting the experiment. Animals were divided into four groups, each of six animals. The control group were given normal saline (10 ml/kg) i.p, the standard group were treated with castor oil (0.2ml/mice) as the standard drug, and the remaining groups were treated with methanolic plant extracts (200 and 400 mg/kg i.p). After 30 min of injecting saline, castor oil and extracts, 10% active charcoal in 100 ml of 5% aqueous gum acacia was administered (5 ml/kg p.o). After 1hr of administering charcoal, the animal was killed by cervical dislocation and dissected. The dissected animals were placed on a clean surface, and the distance travelled by charcoal was measured. Then GIT motility was calculated for all groups. The percent motility was calculated using the following formula:

$$\% \text{ inhibition of mortality} = \frac{T0 - T1}{T0} \times 100$$

T_0 = total length of intestine
T_1 = distance travelled by charcoal in the intestine.

Statistical analysis

The data obtained by the various parameters were statistically evaluated using a one-way variance analysis (ANOVA), followed by a student t-test.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical screening found that flower extract contains alkaloids, phytosterols, diterpenes, aminoacid and proteins, and flavonoids and phenolic compounds. (Table .1).

Table 1.: Results of different group tests of flowers of O.*robusta*

Phytochemicals	Leaves Extract
Alkaloids	-
Phytosterols	+
Diterpins andTriterpens	++
Amino acid and protein	-
Flavonoids	+
Phenolic compounds	+
Saponins	+
Glycosides	+

*+= Presence, - = Absence

Acute Toxicity Test

The *O. robusta* leaves extracts at doses of 1000, 1500 and 2000 mg/kg had no adverse effect on the behavioural responses of the tested mice up to 72hrs of observation. There was no mortality observed at all the tested doses. None of the used doses affected the weight of the mice. Therefore, the LD50 of ORE was estimated to be more than 2000 mg/kg. As the maximal dose used in the analgesic trial was five times less than the doses (200 and 400 mg/kg) administered to mice and rats in the acute toxicity study, it is likely that these doses are exceedingly safe.

Antioxidant Activity

Determination of total phenolic content

The total phenolic content of O. robusta leaf methanolic extracts is shown in Table 2. The total phenol content of O. robusta leaf methanolic extract was determined (using a standard reference curve) at 88.83±1.02 mg of GAE/g of extract, suggesting that it may have antioxidant properties.

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Table 2. Determination of total phenolic contents of *O. robusta* leaves extract.

Extract	Absorbance At 760 nm	Average absorbance.	Total phenolic Mg of GAE/mg of extracts
Methanolic extract	0.456 0.462 0.469	0.4623±.00376	88.83±1.02

Data represent mean ± SEM (n=3) of duplicate analysis

Free radical scavenging activity

In the current study, the fruit methanolic extract showed free radical scavenging activity with an IC_{50} value of 2.67±0.016 mg/ml, and the maximum inhibition was found as 71.83%. On the other hand, the standard Butylated Hydroxy Anisole (BHA) showed maximum inhibition of 93.09 %, and 50% inhibitory concentration (IC_{50}) was found as 4.10±0.035 mg/ml. Figure 1 shows the scavenging activity of leaf extract in a good way.

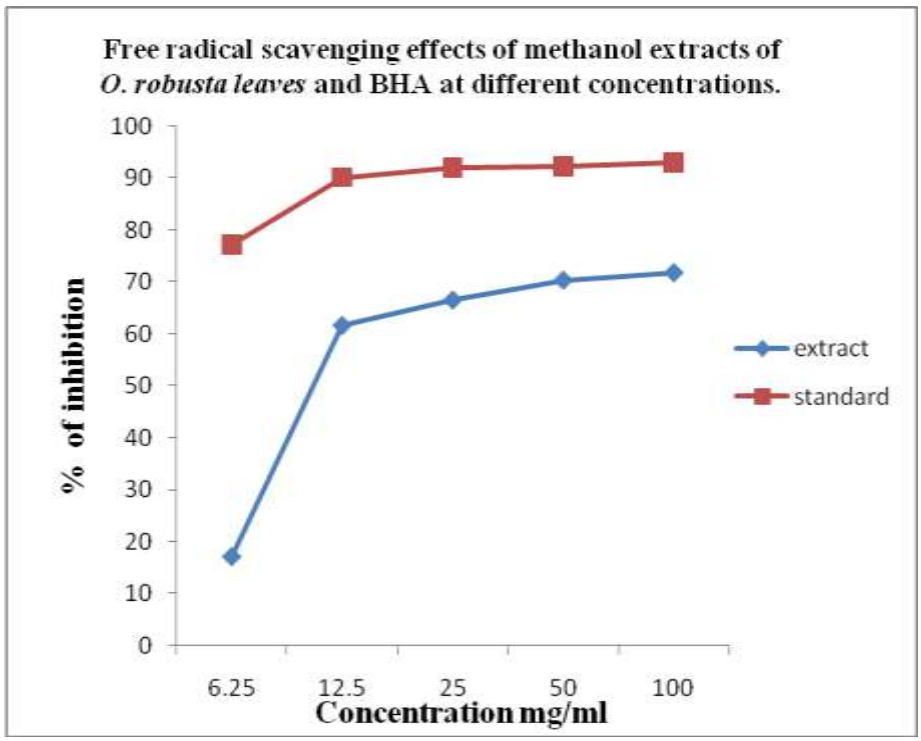

Figure 1: Free radical scavenging effects of methanolic extracts of *O. robusta* and BHA at different concentrations.

Membrane stabilizing activity.

The methanolic leaves extract was subjected to membrane stabilizing activity experiments, and the findings were statistically presented in Table 3. The results showed that the leaf extracts (at a concentration of 1 mg/ml) were significantly ($p<0.001$) effective in human erythrocytes against hypotonic solution and heat-induced lyses compared to the standard drug acetylsalicylic acid (0.10 mg/ml).

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Table 3: Effect of *O. robusta* leaf extract on the hypotonic solution and heat-induced erythrocyte membrane haemolysis.

Treatment	Conc. mg/ml	% inhibition of haemolysis	
		Hypotonic solution-induced hemolysis (Mean±SD)	Heat-induced hemolysis (Mean±SD)
Control	50mM	-	-
ME	1 mg/mL	50.40±3.78***	22.72±3.208***
Acetyl salicylic acid(ASA)	0.10 mg/mL	76.60±3.97***	37.8±3.42***

ME= Methanolic extract, ASA= Acetylsalicylic Acid. Each value is presented as mean ± SEM (n = 5). Data are found to be significant by testing through one-way ANOVA at a 5% level of significance *** p<0.0001 when compared to the control.

Isoniazid induced seizures

Isoniazid (300 mg/kg i.p.) elicited clonic tonic convulsion in all the animals used. The methanolic extract of *O. robusta* leaves (200 mg/kg and 400 mg/kg i.p.) significantly (p<0.01;

0.001) delayed the onset clonic of convulsion time, significantly decreased the duration of isoniazid-induced seizures (p<0.001) and showed a dose-dependent increase in anticonvulsant activity. Similarly, the standard anticonvulsant drug, Diazepam 5 mg/kg i.p., totally abolished the effects of isoniazid-induced convulsion in mice. Both 200mg/kg and 400mg/kg extracts showed 100% protection against INH-induced convulsion in mice with reduced flexion, hind limb extension and stupor phase of convulsion compared to the control treated group. (Table 4).

Table 4: Effects of pre-treated *O. robusta Baker* on Isoniazid-induced convulsions in mice.

Experimental group	Dose mg/kg b.w.	Onset of clonic Convulsion time (sec) Mean±S.D	Duration of convulsion time (sec) Mean±S.D	% Protection
G-I control	10 ml/kg (p.o.)	174.6±31.46	295.00±25.05	0
G-II (Diazepam)	5.0 mg/kg i.p.)	0.00±0.00***	0.00±0.00***	100
G-III ME	200 mg/kg (i.p.)	403.80±157.00**	128.60±19.34***	100
G-IV ME	400 mg/kg (i.p.)	735.25±256.48***	65.40±23.02***	100

All values are expressed as Mean ±S.D, n=5 mice in each group, by one-way ANOVA followed by Dunnett's Multiple Comparisons Test. (compared with the control group) *p<0.05, **p<0.01 and *** p<0.001.

Pilocarpine induced seizures.

Pilocarpine (240mg/kg body weight) produced convulsions in 100% of the mice used. The methanolic of *O. robusta* leaves extract at a dose of 200 mg/kg and 400 mg/kg i.p. significantly delayed the onset of Pilocarpine -induced seizures (p<0.01; 0.001) and significantly decreased the duration of isoniazid-induced seizures (p<0.001). Similarly, the standard anticonvulsant drug, Diazepam 5 mg/kg i.p., totally abolished the effects of Pilocarpine-induced convulsion in mice (Table 5).

Table 5: Effects of methanolic extract on Pilocarpine induced convulsions in mice.

Experimental group	Dose mg/kg Bodyweight	Onset of clonic convulsion (sec) Mean±S.D	Duration of convulsion (sec) Mean±S.D	% Protection
G-I control	20 ml/kg (p.o.)	128.80±37.32	254.06±229.21	0
G_II (Diazepam)	5.0 mg/kg (i.p.)	0.00±0.00***	0.00±0.00***	100
G-III ME	200 mg/kg (i.p.)	319.2±75.044***	144.00±22.32**	100
G-IV ME	400 mg/kg (i.p.)	435.38±109.61***	74.20±32.72**	100

All values are expressed as Mean ±S. D, n=5 mice in each group, by one-way ANOVA followed by Dunnett's Multiple Comparisons Test. (compared with the control group) *P<0.05, **P<0.01 and *** P<0.001.

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GI motility

Both concentrations of methanol extract significantly ($p<0.001$) reduced the distance the charcoal meal travelled through the mice's gastrointestinal tract compared to the control group and the castor oil-treated group. The passage of charcoal meal through the digestive tract was significantly slowed by extract at doses of 200 and 400 mg/kg (53.58% and 55.23%, respectively) (Table 6).

Table 6: GIT motility activity of the extract of *Ormosia robusta*

Treatment	Dose	Total length of intestine Mean±SD	Distance covered by charcoal. Mean±SD	% Motility Inhibition
Control	10ml/kg	61.83±1.77	45.83±1.57	32
Castor oil	0.2ml/mice	50±0.57	.39.58±1.00***	20.84***
extract	200 mg/kg	68.58±2.28	31.83±0.87***	53.58***
	400 mg/kg	60.5±1.11	27.08 ±0.37***	55.23***

Values are expressed as mean±SEM (*n*=5). ***p<0.001 when compared to controls.

DISCUSSION

Some previous studies demonstrated that high levels of total phenolic contents exhibit high antioxidant capacity (S. A. Baba and Malik 2014). Antioxidants with phenolic components are among the most potent kinds of synthetic chain-breaking antioxidants because of their free radical scavenging ability facilitated by their hydroxyl groups, and the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. It also has an important role in preventing lipid oxidation (Sannigrahi et al. 2010). In our study, we also found the methanolic extract of *O. robusta* leaves rich in total phenolic components. The DPPH radical scavenging method is another standard procedure applied to the evaluation of the antiradical activity. The methanolic extract showed dose-dependent DPPH radical scavenging activity. The effect of the free radical scavenging activity of extracts on DPPH radicals is thought to be due to their hydrogen donation ability of polyphenols of *O. robusta*. The results showed that the leaf extract is a free radical scavenger which may limit the occurrence of free radical damage in the human body (Kumarappan, Thilagam, and Mandal 2012). The extract contains other phytochemicals like flavonoids, which suppress reactive oxygen formation, scavenge reactive species, and up-regulate and protect antioxidant defences (S. a. Baba and Malik 2014). Therefore, it may be said that the presence of higher total phenolic components and flavonoids may be responsible for demonstrating the antioxidant activity and free radical scavenging ability of the plant.

The results showed that *O. robusta* extracts were potent in membrane stabilizing activities on human erythrocytes. The activity was comparable to standard anti-inflammatory drugs (aspirin) and control. It was found that anti-inflammatory drugs act either by inhibiting lysosomal enzymes or through the stabilization of lysosomal membranes (I. Khan et al. 2009) as human RBC membranes and lysosomal membrane components are considered similar, so membrane stabilizing activity can play a significant role in anti-inflammatory activity (M. S. S. Khan et al. 2013). It has been previously reported that some phytochemicals like flavonoids exert stabilizing effects on lysosomes (Sadique, J., Al-Rqobah, W.A., Bugharlth, M.E., Gindy 1989). Tannin and saponins also elicited membrane stabilizing activities by binding to the erythrocyte membranes with consequent alteration of surface charges like cations of cells (Shanbrany *et al*., 1977), and the report says that the leaf extract of *O. robusta* has tannins, saponin, and lots of flavonoids. The current analysis reveals that the membrane stabilizing action of *O. robusta* extracts plays a significant role in the anti-inflammatory activity, possibly as a result of their high flavonoids, tannin, or saponin content, which exhibits considerable clot lysis activity.

The methanolic extract exhibited a significant decrease in a different phase of epileptic seizure against Pilocarpine and isoniazid-induced seizures when compared with the control. Still, it is showing moderate activity when compared to that Diazepam. Isoniazid exerts its convulsive effect by inhibiting GABA synthesis below a critical level in some neurons, thereby leading to CNS excitation and convulsions (Wood and Peesker 1973). Isoniazid-induced seizures were carried out further to confirm the GABA-enhancing activity of the plant extract. The methanolic leaf extract also showed anticonvulsant effects against PILO-induced seizures. PILO, a cholinergic agonist, is widely used in studies of epilepsy as a model of experimentally induced limbic seizures. It is demonstrated that Pilocarpine, acting through muscarinic receptors, causes an imbalance between excitatory and inhibitory transmission resulting in the generation of SE and simultaneously decreasing acetylcholinesterase enzymatic activities and GABAergic receptor densities(Doughari et al. 2012; Giulia, C., Daniela, L., Giuseppe, B., Roland, S.G.J., and Massimo 2008; Quintans et al. 2008). The extract, when compared to the control-treated group, produced a significant increase in the time of onset of clonic seizures; this shows a dose-dependent increase in the anticonvulsant activity in both pilocarpine-evoked status epilepticus (SE) and isoniazid induce a seizure. Diazepam widely used as a convulsion-reducing drug, also did not affect

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the incidence of seizures or death but significantly delayed the onset of seizures.

Previous studies showed that various phytochemicals like terpenoids, alkaloids, saponins, and flavonoids showed anticonvulsant activity (Hui-Ling, Z., Jian-Bo, W., Yi-Tao, W., Bao-Cai 2014). Terpenoids exhibited an anticonvulsive effect (Kazmi et al. 2012). This effect, possibly mediated by the facilitation of GABA transmission, elevates GABA levels, possibly through potentiation of GABA synthesis, which has been evaluated in isoniazid-induced seizures in rats. Terpenoids could reduce pilocarpine-induced seizures probably by modulating other systems' neurotransmitters rather than the GABAergic system (Costa, J.P., Ferreira, P.B., De Sousa, D.P. 2012). Saponins components are useful in the treatment of convulsive disorders, although their action mechanisms remain unclear (Jalsrai, A., Grecksch, G., Becker 2010). Flavonoids, one of the major antioxidants, has been discovered to possess remarkable anticonvulsant and neuroprotective effects on pilocarpine-evoked status epilepticus (SE) and isoniazid induce a seizure in rats (Diniz et al. 2015). There is ample evidence to show that lipid peroxidation levels are increased during the acute period of PILO-induced seizures in adult rats (Tejada, S., Sureda, A., Roca, C., Gamundí, A., Esteban 2007), suggesting the involvement of free radicals in the PILO-induced brain damage. This is further supported by the fact that certain antioxidants have shown anticonvulsant activity against PILO-induced SE, which could be attributed to cholinergic antagonism at the M1 or M2 receptors or an increase in GABA and/or/or its receptor densities or through antioxidant mechanisms (Carlos, R.M., Carmen, A., José Luis, G.G., José Santiago, I.C., Marta, SC, Federico 2014). Data from our laboratory (antioxidant test) indicate that ME has a marked antioxidant and free radical scavenging effect *in vitro*. And the anticonvulsant effect may be ascribed to the protection of endogenous enzyme levels, an increase of the GABA level in the brain, and inhibition of oxidative injury.

The charcoal meal passage test is one of the well-established methods for finding GIT motility in animal models. It is well-established that castor oil produces diarrhea by the release of prostaglandins which stimulate the gastrointestinal motility and secretion of water and electrolytes (Murugesan, T., Ghosh, L., Mukherjee, K., Das, J., Pal, M., Saha 2000). Our results indicate that the ME decreased gastrointestinal motility was comparable to control and castor oil as a standard drug. Various researchers have suggested the presence of phytoconstituents in the plant leaves, like flavonoids, tannins, saponins, polyphenols, alkaloids and reducing sugars, can decrease GI motility (Atta, A.H., Mouneir 2004; Sebai, H., Jabri, M.A., Souli, A., Rtibi, K., Selmi, S., Tebourbi, O., El-Benna, J., Sakly 2014; Umer, Tekewe, and Kebede 2013)**.** The dose-dependent activity of ME extract can be possibly attributed to the dose-dependent actions of flavonoids (Jeffrey B. Harborne and Williams 2000). Various researchers reported that tannins, polyphenols, reducing sugars, and saponins (Ojewole, J.A., Awe, E.O., Nyinawumuntu 2009) could be responsible for antidiarrheal actions. The plant is good for constipating as it decreases intestinal motility. Thus, antidiarrheal actions shown by ME extract may be possibly correlated with the presence of such phytochemicals.

CONCLUSION

The crude extract of leaves is used to cure constipation in traditional medicine, and the extraction of such chemicals can aid in developing antidiarrheal drugs. The hydro-ethanolic extract of the entire plant of *O. robusta* exhibits anticonvulsant and motor impairment effects in rodents, most likely due to an interaction with gabaergic neurotransmission. Antioxidant characteristics may alter anticonvulsant effects. This study proves that this herb has been traditionally used to treat epilepsy in Ghana. Future research will isolate, purify, and identify the chemical components responsible for the CNS actions of *O. robusta*.

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COMPETING INTERESTS

There are no conflicts of interest associated with the authors of this paper.

AUTHORS' CONTRIBUTIONS

All of the authors collaborated to finish this work. PC conceived the study, performed the statistical analysis, produced the protocol, and wrote the report. FM did the literature searches. LB did the analysis and supervised study. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

All animal experiments were performed in accordance with the Animal Ethical Committee from Noakhali Science and Technology Research (NSTU) Cell of Noakhali Science and Technology University, Bangladesh. The animal experiments were reviewed and approved by Animal Ethics Committee of Noakhali Science and Technology University, Bangladesh.

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