

Cytotoxicity and Sedative Activity of Steam Bark of *Dillenia indica* L.

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(Received: April 12, 2020; Accepted: June 10, 2021; Published (Web): July 15, 2021)

Abstract

The study aims to assess the cytotoxic activity of the methanolic extract (CME) and various fractions from the bark of *Dillenia indica* L (Family: Dilleniaceae) using brine shrimp lethality bioassay and sedative activities using hole cross, open field, and elevated-plus maze (EPM) test in Swiss albino mice. In brine shrimp assay, the LC₅₀ for CME, pet-ether (PETF), chloroform (CHF) and aqueous (AQF) fraction were found to be 110, 24.55, 85 and 14.45 µg/ml, respectively, indicating significant cytotoxicity of PETF and AQF when compared to the standard vincristine sulfate (7.5 µg/ml). In open field test, the number of movements per min (after 120 min) was 40.90, 12.43, 5.45, 3.66, and 29.74 for control, standard diazepam, CME, CHF and AQF, respectively, indicating strong activity of the chloroform soluble fraction compared to the other test samples of *D. indica*. Similar data was observed in hole cross test where the number of movements per minute after 120 min were 3.60, 5.56, 4.77, and 7.99 for the standard, CME, CHF and AQF, respectively. In EPM test, the CME showed a significant decreased percentage of entries of mice into the open arms of EPM as well as the percentage of time spent in the open arms. The result indicates that the CHF significantly suppressed the locomotor activity than that of methanol and aqueous fractions. These findings will be helpful for bioassay-guided isolation of active principles responsible for cytotoxicity and sedative activities.

Key words: *Dillenia indica*, Dilleniaceae, Cytotoxicity, Sedative, Maze test, Hole cross test.

Introduction

Plants have been serving as excellent resources to unveil a variety of pharmacological actions over many decades (De Silva, 1997; Verpoorte, 1998). It is estimated that about 80% of world population depends mainly on plant-based drugs (Jackson *et al.*, 2012) and in Bangladesh, about 75% of the populations rely on different forms of traditional medicines for their primary health care (Haque *et al.*, 2018). To explore the potential of traditional medicinal plants available in Bangladesh, we selected *Dillenia indica* L (elephant apple, Family: Dilleniaceae), locally known as Chulta. The plant is an evergreen edible species widely grown in the

tropical forests in Bangladesh and is originated from Indonesia (Alam *et al.*, 2011). Traditionally, the plant has been used for a long time against cancerous growth, cough, constipation, diabetes, fever, jaundice, laxative, stomachache, snake bite, etc. (Saiful, Y.L and Armania, N., 2014). According to literature review, a wide variety of biological activities have been reported from the leaf, bark and fruit of this plant including antioxidative (Abdille, 2005), anti-diarrheal and anti-leukemic (Sharma *et al.*, 2001), cardiotoxic effect (Kumar *et al.*, 2010), and anti-inflammatory and CNS depressant (Alam *et al.*, 2011; Mohona *et al.*, 2016) effects, etc.

Considering the traditional uses and extensive

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DOI: <https://doi.org/10.3329/bpj.v24i2.54708>

literature review of *D. indica*, we selected the plant to find out the cytotoxic activity and CNS depressant properties of the methanolic crude extract and its different solvent fractions using brine shrimp lethality bioassay, and hole cross, open field, and elevated-plus maze (EPM) tests in mice.

Materials and Methods

Collection and identification of plants: The bark of *Dillenia indica* were collected from Dinajpur district, Bangladesh during the month of November 2015, and the plant was identified by Dr. A H M Mahbubur Rahman, Professor and taxonomist, Department of Botany, University of Rajshahi, Bangladesh. A voucher specimen for this collection has been deposited in Jahangirnagar University Herbarium (JUH/32234).

Extraction and fractionation: The collected barks were first washed with water to remove adhering dirt and then shade dried for several days with occasional sun drying followed by oven drying for 24 hours at 45°C. The dried materials were crushed into a coarse powder. About 100 gm powdered materials were taken in an amber-colored glass bottle and were soaked with methanol (100 mL × 3 times) for 7 days with occasional shaking. The extract (CME) was filtered through a cotton bed followed by Whatman No. 1 filter paper, concentrated with a rotary evaporator under reduced pressure and preserved at 4 °C. After dilution with adequate amount of water, the CME was partitioned with petroleum ether, and chloroform to get petroleum ether (PETF), chloroform (CHF), and aqueous (AQF) soluble fractions.

Cytotoxicity assay using brine shrimp lethality bioassay: The experiment was carried out by the method described by Meyer *et al.* (1982). In brief, *Artemia salina* Lech. (brine shrimp eggs) were allowed to hatch for 48 hours in simulated sea water and allowed to mature as nauplii (Larvae) at 25 °C. Serially diluted samples dissolved in DMSO from each of CME, PETF, CHF, and AQF were added to 5 ml simulated sea-water containing 20 nauplii. Then 120 µl of DMSO was added to each of the three pre-

marked glass vials containing 5 ml of simulated sea water and 20 shrimp nauplii to use as negative control group. After 24 hours incubation at 25°C, the number of survivors was counted. The LC₅₀ (50% lethal concentration in µg/ml) values were determined from triplicate experiments. Different concentrations of vincristine sulfate were taken as positive control.

Animals: Swiss albino mice (weight 32-37 gm) of both sexes were collected from the International Centre for Diarrheal Disease and Research, Bangladesh (icddr,b). The animals were housed in plastic cages having dimension of 28×22×13 cm under standard laboratory conditions (relative humidity 55-65%, room temperature 23.0±2.0°C and 12 hours light : dark cycle) and acclimatized for 7 days and were fed with standard diet and water. The ethical guidelines for the investigation of experimental animals were followed in all tests (Bowd, 1980).

Sedative activity: Three methods were used to investigate the sedative activity of the extracts: open field, hole cross and elevated plus maze (EPM) tests, the experimental details of which have been described in our previous paper (Ali *et al.*, 2014). In brief, the animals were divided into control, standard and experimental groups containing five mice in each group. The control group received 10 ml/kg 1% Tween 80 in water, the experimental groups received extractives (CME, CHF, and AQF) at a dose of 400 mg/kg body weight and the standard group received standard diazepam at a dose of 1 mg/kg body weight.

Open field test: This experiment was carried out according to the method described by Gupta *et al.* (1971). The apparatus had a wall of 40 cm high. The number of squares visited by the mice was counted for 3 min, on 0, 30, 60, 90 and 120 min during the study period.

Hole cross test: The protocol described by Takagi *et al.* (1971) was adopted to the test CNS depressant activity in mice. The total number of passages of a mouse through the hole from one chamber to another was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral administration with test substances.

Elevated plus maze (EPM) test: The method described by Lister (1987) utilizing an appearance consisting of two open arms (5×10 cm) and two closed arms (5×10×15 cm) radiating from a platform (5×5 cm) to give a plus sign in appearance was used. The maze floor and walls were made with dark opaque wood. Sixteen minutes after administration of the test agents, each animal was placed at the center of the maze facing one of the enclosed arms. During the five min test period, the number of open arms entries was recorded. The entry into an arm was defined as the point when the animal places all four paws onto the arm.

Statistical analysis: All data were expressed as mean ± STD and were analyzed by one way ANOVA followed by using Dunnett's test. The difference was considered significant at $p < 0.05$.

Results and Discussion

Brine shrimp lethality bioassay: The methanol extract (CME) of *D. indica* and its three fractions (PETF, CHF and AQF) were evaluated for lethality against brine shrimp nauplii (Meyer et al., 1982). It

was observed that the mortality rate of nauplii varied with concentration of sample and was increased with increasing concentration. The results expressed as median lethal concentration (LC_{50} in $\mu\text{g/ml}$) have been shown in Figure 1. The LC_{50} was determined by extrapolation from graph and the values for CME, PETF, CHF, AQF and standard vincristine sulfate were found to be 110, 24.55, 85, 14.45, and 7.5 $\mu\text{g/ml}$, respectively. Compared to positive control, the results indicate that aqueous as well as pet ether soluble fractions showed strong lethality against brine shrimp nauplii (Figure 1). Our data is in line with that of previous reports (Alam et al., 2011) except the activity of aqueous soluble material (AQF), where the authors reported inactivity of AQF ($>200 \mu\text{g/ml}$), instead of potent activity (14.45 $\mu\text{g/ml}$). Since, the brine shrimp lethality assay is a useful tool for preliminary toxicity evaluation of natural products (Carballo et al., 2002), the findings has the potential to rationalize the use of the plant materials in traditional medicine against cancerous growth.

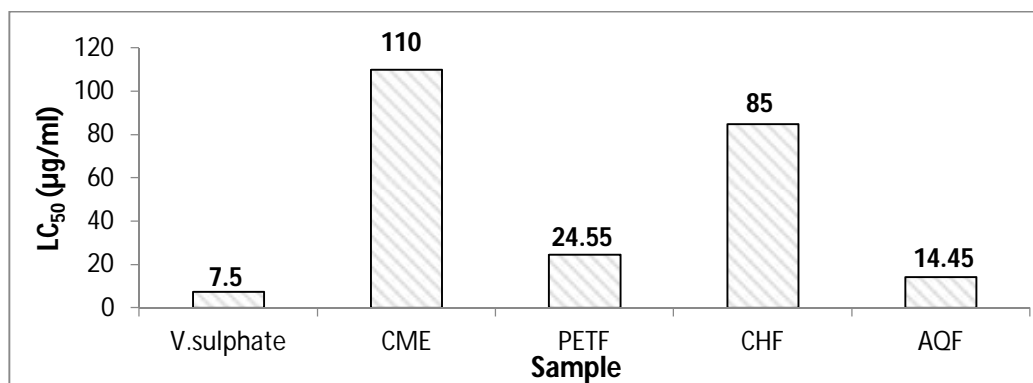


Figure 1. Results of brine shrimp lethality bioassay of crude extract and fractions of *D. indica*.

Sedative activity

Open field test: In this test, the number of squares traveled by the mice was suppressed significantly throughout the study period after administration of the extracts at a dose of 400 mg/kg bw. About 120 min after the administration of samples, the number of movements per min was

40.90, 12.43, 5.45, 3.66, and 29.74 for control, standard diazepam, CME, CHF and AQF, respectively, indicating the strong activity of chloroform soluble fraction compared to the other fractions. The significant decrease in locomotion was dose-dependent as depicted in Table 1. The CNS depressant activity obtained for the extractives was

also statistically significant. Similar activity of methanolic extract was previously reported by Mohona *et al.* (2016) and we found the highest activity of chloroform fraction. Open field test is a

common measure of altered behavior and general activity, where both the qualitative and quantitative analysis of rodent behavior can be determined.

Table 1. Sedative activity of crude methanolic extract and various fractions from *D. indica* using open field test in mice model.

Group	Treatment	Numbers of square travelled				
		0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in water	64.66±6.55	49.32±4.05	40.00±4.22	36.22±3.22	40.90±3.60
Standard	Diazepam	66.23±2.05	44.90±3.22	29.00±4.15*	10.03±6.12*	12.43±2.55*
Test sample	CME	63.56±6.70	14.55±6.22*	9.00±5.42	4.45±8.43*	5.45±6.45*
	CHF	66.46±6.70	55.78±3.41*	22.44±2.42	9.88±4.56*	3.66±8.89*
	AQF	62.55±6.70	56.66±7.86*	37.65±6.88*	30.48±5.57	29.74±7.66*

Values are expressed as mean ± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunntt's test; *p<0.05, significant compared to control. Dose for control, standard and extracts was 10 mg/kg bw (p.o), 1 mg/kg (i.p.) and 400 mg/kg (p.o), respectively.

Hole cross test: In the hole cross test, the total number of hole crossed by mice from one chamber to another was counted. The number of movements after 120 min were 3.60, 5.56, 4.77, and 7.99 per minute for standard diazepam, CME, CHF and AQF, respectively (Table 2), indicating a significant decreased locomotor activity of the extract at 400

mg/kg bw as compared to the control group. The result was statistically significant (p<0.05). Similar data for methanolic fraction was reported by Mohona *et al.* (2016). In our report, the activity of chloroform fraction that is higher than that of methanol and aqueous fraction is the new finding.

Table 2. Sedative activity of crude methanolic extract and various fractions from *D. indica* using hole cross test in mice model.

Group	Treatment	Numbers of hole crossed				
		0 min	30 min	60 min	90 min	120 min
Control	1% Tween 80 in water	17.55±3.22	16.00±1.98	12.44±2.13	8.74±2.55	6.45±1.612
Standard	Diazepam	19.82±3.47	10.46±4.22	8.54±3.42*	5.45±1.46*	3.60±2.22*
Test sample	CME	15.54±3.24*	7.44±5.14*	4.42±0.84*	9.69±0.46*	5.56±2.32*
	CHF	19.47±3.32*	15.88±6.22*	14.78±5.54*	6.55±2.33	4.77±3.42*
	AQF	16.85±3.22*	16.23±5.44	12.77±2.54	8.44±4.33*	7.99±6.22*

Values are expressed as mean± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunntt's test; *p<0.05, significant compared to control. Dose for control, standard and extracts were 10 ml/kg bw (p.o), 1 mg/kg bw (i.p.) and 400 mg/kg bw (p.o), respectively.

Elevated plus maze (EPM) test: In EPM test, the crude methanolic extract at a dose of 400 mg/kg body weight showed a significant decreased percentage of

entries of mice into the open arms of EPM as well as the percentage of time spent in the open arms (Table 3). EPM test is a suitable model for testing specific

GABA receptor related activity such as benzodiazepines or GABAA (γ -Aminobutyric acid type A) agonists, but not for other drugs (Engin and

Treit, 2008). In our experiment, we found that the activity of crude methanolic extract was comparable to that of the anxiolytic agent, diazepam.

Table 3. Sedative activity of crude methanolic extract and various fractions from *D. indica* using elevated plus maze test in mice model.

Group	Treatment	Entry into open arm (%)	Time spent in open arm (%)
Control	1% Tween 80 in water	65.44±4.66	50.45±5.36
Standard	Diazepam	38.11± 5.44*	37.27±2.53*
Test sample	CME	35.83±7.25*	18.76±5.42*
	CHF	58.54±3.77*	44.98±2.77*
	AQF	63.48±4.54*	49.65±4.52*

Values are expressed as mean± STD (n=5); One way Analysis of Variance (ANOVA) followed by Dunnett's test; *p<0.05, significant compared to control. Dose for control, standard and extracts were 10 ml/kg bw (p.o), 1 mg/kg bw (i.p.) and 400 mg/kg bw (p.o), respectively.

Conclusion

D. indica is one of the important species of Dilleniaceae family which exhibits plethora of pharmacological activities. In addition to anti-laxative, astringent, antimicrobial, antidiabetic and anti-oxidant activities (Boparai et al., 2016), various part of the plant have been reported to possess CNS depressant (Alam et al., 2011; Mohona et al., 2016; Singh et al., 2016) and suppression of tumor cell proliferation (Sharma et al., 2001) activities. Our reports also justify the use of this plant in folklore medicine for the management of depression and tumor cell proliferation.

In conclusion, our findings with significant cytotoxicity of pet-ether and aqueous soluble fractions of methanol extract of bark of *D. indica* and the sedative activity of its chloroform fraction may provide valuable information for bioassay guided isolation of active principle responsible for the particular activities.

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