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## Research Article

### BIOLOGICAL ACTIVITIES OF *DILLENIA INDICA* L. BARK GROWING IN BANGLADESH

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*Correspondence	Abstract
<p>Md. Mizanur Rahman Moghal Assistant Professor, Department of Pharmacy, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh</p> <p><b>DOI: 10.7897/2321-6328.01202</b></p> <p>Article Received on: 23/06/13 Accepted on: 27/07/13</p>	<p>The study was designed to explore possible phytochemical characteristics (group determinant of plant extract) and to investigate the cytotoxic and anthelmintic potential <i>in vitro</i>, using the methanolic extract of bark of <i>Dillenia indica</i> growing in coastal region of Bangladesh. Phytochemical analysis of the crude extract revealed the presence of alkaloid(s), carbohydrate(s), glycoside(s), phenol, tannin, protein(s), gum and mucilages. N-hexane and ethyl acetate soluble fraction of <i>Dillenia indica</i> were screened for cytotoxic activity using brine shrimp lethality bioassay, where vincristine sulphate was used as a positive control. It was noticed that the methanolic extract and its fractions possess potent cytotoxic principles (with LC<sub>50</sub> value 17.68 µg / ml, 17.68 µg / ml, 15.80 µg / ml and LC<sub>90</sub> value 486.61, 287.66, 148.82 µg / ml respectively) compared with positive control vincristine sulphate (LC<sub>50</sub> 0.631 µg / ml and LC<sub>90</sub> value 13.51 µg / ml). The other study was undertaken to evaluate anthelmintic activity where albendazole was used as reference standard. Methanolic extract of barks (25 mg / mL) caused paralysis of the worms at 136 minutes and death at 176.0 minutes while albendazole (positive control) paralyzed and killed the worms at 17.67 minutes and 48 minutes, respectively. The study confirms the mild anthelmintic activity of bark extract of <i>Dillenia indica</i> and therefore suggests the isolation of active principles through bioassay.</p> <p><b>Keywords:</b> <i>Dillenia indica</i>, Anthelmintic activity, Cytotoxic activity, Phytochemical screening, LC<sub>90</sub></p>

## INTRODUCTION

As natural sources, medicinal plants play an important role for most medicinal preparations such as plant materials, refined petroleum extracts and mixtures, etc. Even in recent times, most people still depend on traditional medicines for their primary health care. According to the World Health Organization, nearly 80 % of the world population still relies on traditional herbal medicines<sup>1</sup>. There is evidence that fruits and herbs contain phytochemicals and nutrients can protect the human rights of a number of diseases by their biological activities<sup>2</sup>. *Dillenia indica* L. is a large shrub or small to medium-sized tree growing to 15 m tall under the family Dilleniaceae, somewhere known as elephant apple and locally known as Chalta. It is an ethno-medicinally important plant used for the treatment of severe diseases like cancer and diarrhea<sup>3</sup>. This plant was selected due to its availability in Bangladesh is huge, therefore, lots of people in the rural area use this plant for different treatments and not much investigations has been carried out with this plant of this region. Our main goals were to evaluate the possible chemical groups and investigate the cytotoxic and anthelmintic properties of *Dillenia indica* bark to validate its traditional uses.

## MATERIALS AND METHODS

### Plant material collection

The bark of *Dillenia indica* was collected by the authors from the surrounding area of Noakhali, a coastal region of

Bangladesh in September, 2011. The plant was identified and authenticated by expert botanist of Bangladesh National Herbarium (DACB), Mirpur, Dhaka and a voucher (Accession no. 35652) has been deposited to the herbarium for future reference.

### Crude extraction

Weighed 330 g of the dried and powdered sample was soaked in 1300 ml of 80.0 % methanol (Merck KGaA, Germany). After 15 days the solution was filtered using filter cloth and Whatman® filter paper No. 1. The resulting filtrates were then evaporated in water bath maintained at 40°C to dryness and thus a blackish semisolid mass of the extract was obtained.

### Partitioning with n-hexane

The mother solution was taken in a separating funnel. 100 ml of the n-hexane was added to it and the funnel was shaken and then kept undisturbed (Figure 1). The organic portion was collected. The process was repeated thrice; n-hexane fractions were collected together and evaporated. The aqueous fraction was preserved for the next step.

### Partitioning with ethyl acetate

To the mother solution left after washing with n-hexane, 12.5 ml of distilled water was added and mixed. The mother solution was then taken in a separating funnel and extracted with ethyl acetate (100 ml X 3) (Figure 2). The ethyl acetate

fractions were collected together and evaporated. The aqueous fraction was preserved for the next step.

#### Phytochemical evaluation

Small quantity of freshly prepared methanolic extract of *Dillenia indica* bark were subjected to preliminary quantitative phytochemical investigation for the detection of phytochemicals such as alkaloids, carbohydrates, glycosides, phytosterols, proteins, flavonoids, tannins, saponins, phenols, gums and mucilages, fats and fixed oils using the following standard methods<sup>4,5</sup>.

#### Detection of alkaloids

Extract was dissolved in dilute hydrochloric acid and the solutions were filtered.

- a) **Mayer's Test:** Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate marked the presence of alkaloids.
- b) **Hager's Test:** Filtered solution was taken in a test tube and Hager's reagent (saturated picric acid solution) was added with it. Presence of alkaloids was confirmed by the formation of yellow colored precipitate.

#### Detection of carbohydrates

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was evaluated for the presence of carbohydrates.

- a) **Benedict's test:** Filtrate was treated with Benedict's reagent and heated gently. Orange red precipitate pointed the presence of reducing sugars.
- b) **Fehling's Test:** Filtered solution was hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate specified the presence of reducing sugars.

#### Detection of glycosides

Extract was hydrolyzed with dil. HCl, and then subjected to test for glycosides.

- a) **Legal's Test:** Extract was mixed with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicated the presence of glycosides.
- b) **Modified Borntrager's Test:** Extract was treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammoniacal layer showed the presence of glycosides.

#### Detection of saponins

- a) **Froth Test:** Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam expressed the presence of saponins.
- b) **Foam Test:** 0.5 g of extract was shaken with 2 ml of water. Foam was produced which remained for 10 minutes and pointed the presence of saponins.

#### Detection of phytosterols

- a) **Salkowski's Test:** Extract was treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand.

Appearance of golden yellow color showed the presence of triterpenes.

- b) **Liebermann Burchard's test:** Extract was mixed with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled and then conc. sulphuric acid was added. Formation of brown ring at the junction confirmed the presence of phytosterols.

#### Detection of phenols

**Ferric Chloride Test:** Extract solution was taken in test tubes and 3-4 drops of ferric chloride solution were added to them. Formation of bluish black color indicated the presence of phenols.

#### Detection of tannins

**Gelatin Test:** To the extract, 1 % gelatin solution containing sodium chloride was added. Formation of white precipitate confirmed the presence of tannins.

#### Detection of flavonoids

- a) **Alkaline Reagent Test:** Extract was treated with 4-5 drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.
- b) **Lead acetate Test:** 4-5 drops of lead acetate solution was added to the extract solution. Formation of yellow color precipitate marked the presence of flavonoids.

#### Detection of proteins and amino acids

- a) **Xanthoproteic Test:** The extract was treated with 4-5 drops of conc. Nitric acid. Formation of yellow color indicated the presence of proteins.
- b) **Ninhydrin Test:** To the extract, 0.25 % w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicated the presence of amino acid.

#### Detection of fixed oils and fats

A few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of extract along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 h. Formation of soap or partial neutralization of alkali pointed the presence of fixed oils and fats.

#### Detection of gums and mucilages

1 ml of the extract was hydrolyzed using dil. HCl (3 ml). Then Fehling's solution was added drop by drop till the appearance of red. Test for mucilages were carried out by treating 1 ml of extract with 2 ml of ruthenium red solution to get red colored solution.

#### In vitro cytotoxicity study

The cytotoxic activity of the extracts was examined using brine shrimp lethality bioassay<sup>6</sup>. In this study vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 40 µg / ml from which serial dilutions were made using DMSO to get 20 µg / ml, 10 µg / ml, 5 µg / ml, 2.5 µg / ml, 1.25 µg / ml, 0.625 µg / ml, 0.3125 µg / ml, 0.15625 µg / ml and 0.78125 µg / ml solution from all three extracts. Then the positive control solutions were added to the pre-marked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups. 100 µl of DMSO was added to each of three pre-

marked glass vials containing 5 ml of simulated sea water and 10 shrimp nauplii to use as control groups.

### Counting of nauplii

After 24 h, by using a magnifying glass, the vials were inspected and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

### In vitro anthelmintic activity study

The anthelmintic study was carried out by the method of Ajaiyeoba *et al.*<sup>7</sup> with minor modifications. Adult earthworms were selected for the study of anthelmintic activity because of their anatomical and physiological resemblance with the intestinal roundworm parasites of human being<sup>8-10</sup>. Earthworms are widely used as effective tools for anthelmintic study due to their availability<sup>11</sup>. Adult

earthworm (*Pheretima posthuma*) were collected (3-5 cm in length and 0.1- 0.2 cm in width weighing about 0.8-3.04 g) from moist soil of a road side field of Noakhali Science and Technology University, Sonapur, Noakhali. All the worms were properly washed with normal saline in order to remove all fecal materials. Extracts were weighed and dissolved in 10 mL of distilled water to obtain the solution of 10, 15, 20, and 25 mg / ml. Albendazole was used as reference standard (10 mg / mL). Earthworms were divided into seven groups (each containing three worms) in petridish. In five groups extract solution was applied, one is for reference and one is for negative control. Observations were made for the determination of paralysis time and death time of the worm. Paralysis was designated as the occurrence where the worms do not move even in normal saline and death was confirmed when the worms lose their motility followed with fading away of their body color.

**Table 1: Phytochemical screening of the methanolic extract of *Dillenia indica* L. bark**

Group of phytoconstituents	Methanolic extract
Alkaloids	+
Carbohydrates	++
Glycosides	+
Saponins	-
Phytosterols	-
Phenols	++
Tannins	+
Flavonoids	-
Proteins and amino acids	+
Fats and fixed oils	-
Gum and mucilages	+

(+) = slightly presence of constituents; (++) = significantly presence of constituents (-) = Absence of constituents

**Table 2: Cytotoxic effect of the test sample of *Dillenia indica***

Sample	LC <sub>50</sub> (µg/ml)	LC <sub>90</sub> (µg/ml)
Vincristine sulphate	0.758	12.77
Crude extract	17.68	486.61
n-hexane fraction	17.68	287.66
Ethyl acetate soluble fraction	15.803	148.82

**Table 3: Anthelmintic activity of crude methanolic extract of bark of *Dillenia indica* against *Pheretima posthuma***

Group	Concentration (mg/ml)	Paralysis time (min)	Death time (min)
		Mean ± SEM	Mean ± SEM
Sample I	10	194 ± 1.7873	237 ± 1.201
Sample II	15	183 ± 1.6583	205 ± 2.127
Sample III	20	149 ± 0.9428	196 ± 1.105
Sample IV	25	136 ± 1.190	176 ± 1.201
Standard	10	17.67 ± 0.54	48 ± 0.47

Here, n = 5, SEM = Standard Error Mean



Figure 1: Partitioning with n-hexane



Figure 2: Partitioning with ethyl acetate

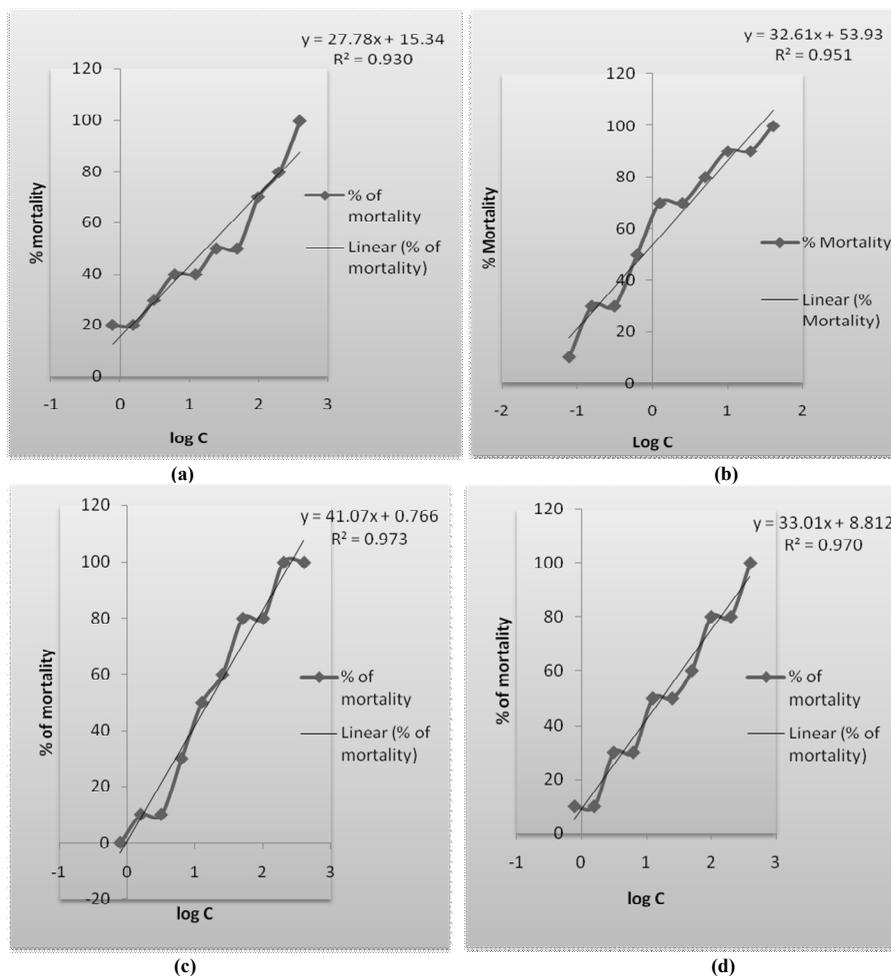


Figure 3: Effect of (a) vincristine sulphate, (b) crude methanolic extract, (c) n-hexane extract, and (d) ethyl acetate extract on brine shrimp nauplii

## RESULTS

### Phytochemical screening

Phytochemical analysis of methanolic extract of bark of *Dillenia indica* revealed the presence of alkaloids, carbohydrates, glycosides, phenols, tannins, proteins, gum and mucilages (Table 1).

### Brine shrimp lethality bioassay

LC<sub>50</sub> (lethal concentration of half of the test organisms) and LC<sub>90</sub> (lethal concentration of 90 % of the test organisms) data (for establishing therapeutic index) of vincristine

sulphate and all three extracts have been given in Table 2 and Figure 3.

### In vitro anthelmintic activity

From the data (Table 3), we see that, the methanolic extract of *Dillenia indica* demonstrated paralysis as well as death of worms in a much more time even in higher concentration.

## DISCUSSION

From the results of the brine shrimp lethality bioassay it can be well predicted that the methanolic extract, n-hexane and ethyl acetate extracts possess potential cytotoxic principles.

Comparison with positive control vincristine signifies that cytotoxicity exhibited by the all three extracts have antitumor activity. Again, crude methanolic extract showed mild anthelmintic activity compared with the positive control. Therefore, these tests validate the folkloric use of *Dillenia indica* L. bark as antitumor and anthelmintic agent. From the above discussion it can be suggested that further *in vivo* investigation is needed to ensure anticancer, analgesic and anti-inflammatory activities of the bark of *Dillenia indica* and also it would be interesting to find out responsible compound(s) and relative mechanisms for the mentioned activities.

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