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Diuretic activity and brine shrimp toxicity of *Derris trifoliata* Lour.

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Abstract

In the present study, ethanol extract of aerial parts of *Derris trifoliata* Lour was evaluated for its diuretic activity by measuring urine volume in mice and general toxicity activity by brine shrimp test (BST) as a part of pharmacological screening of this plant. Compared to control animals, the plant extracts showed significant increment on diuresis, particularly in relation to the total urine excreted in 24 hour. In BST lethality biassay, the plant extract exhibited significant toxicity towards the brine shrimp nauplii. Preliminary phytochemical analyses of the extract indicated the presence of a wide variety of natural product classes that showed significant activities in the bioassays. The overall results suggest that *D. Trifoliate* aerial extracts contain some active principles possessing significant diuretic and toxicity activities.

Key-Words: *Derris trifoliata* Lour, Diuretic activity, Brine shrimp test, Phytochemical analysis

Introduction

Derris trifoliata is an evergreen mangrove plant belonging to the family Fabaceae (Leguminosae) which is commonly known as the legume family, pea family, bean family or pulse family. It is probably the only common climber that grows in mangroves, especially in Sundarban (mangrove forest) of India and Bangladesh. It is a perennial climber, or a much branched climbing shrub, reaching a length of 8 meters or less. The plant has 12.5-20 cm long odd-pinnate compound leaves with 3-7 leaflets (5.7-10 cm by 3.2-5 cm) and 7.5-15 cm long white flowers that are fascicled in axillary racemes.

Several rotenoids [1,2] and glycosidic compounds [3] have been isolated from aerial parts of *D. trifoliata*. Rotenone, one of its principal secondary metabolite has low toxicity to mammals, but is extremely toxic to fish [4]. Some species belonging to the genera including *D. trifoliata* and *D. elliptica*, are commercially cultivated as sources of insecticidal rotenoids[5]. Other species including *D. scandens* and *D. indica* have been traditionally used as diuretic and antidysentery[6].

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However, these therapeutic potentials of the plant have not been scientifically evaluated. Therefore, the present study was undertaken to investigate diuretic activity of the aerial parts` extract of *D. trifoliata* by measuring urine volume in mice and its cytotoxic activity by using brine shrimp lethality bioassay.

Material and Methods

Plant material

The plant *D. trifoliata* was collected from the Koromjol Area of Sundarbon Mangrove Forest during the middle of the July, 2005. The identification of the plant material was confirmed by the experts of Bangladesh National Herbarium, Mirpur, Dhaka and also by the authorities of Botanical Garden, Mirpur, Dhaka. The collected aerial parts of the plant were washed with water and sun-dried for one week after cutting into small pieces. The plant parts were ground into a fine powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an air-tight container and kept in a cool, dark and dry place prior to extraction process.

Preparation of extracts

About 500 gm of powered material was soaked in 1300 mL of 80% ethanol for 7 days accompanying occasional shaking and stirring. The whole mixture was filtrated through white cotton plug followed by Whatman number 1 filter paper. The filtrate thus

obtained was concentrated by using a rotary evaporator (Bibby RE200, Sterilin Ltd., UK). The concentrated extract was then air dried to solid residue (15.5 g) which is treated as the ethanol extract and used for further investigation.

Animals

White albino mice (*Swiss-webstar* strain, body weight = 20-25 gm) of both sexes were purchased from the animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were kept in standard environmental conditions for at least one week for adaptation and had free access to standard laboratory food and drinking water *ad libitum*. All animal experiments were conducted on an isolated and noiseless condition.

Diuretic activity

Prior to the start of the experiment, the mice were fasted overnight with free acces to water. Then, the mice were randomly allocated in individual metabolic cage and divided into two groups consisting of six mice in each group. The first group of animals serving as control, received vehicles only (distilled water containing 0.1% Tween-80) while the second group were treated with suspension of the extract of aerial parts of *D. trifoliata* at an oral dose of 1000 mg/kg body weight. The volume of urine excreted was measured at the end of 1, 2, 4, 8, 12 and 24 h after the treatment.

Brine shrimp bioassay

The plant extracts were evaluated in a test for lethality to brine shrimp larvae (nauplii)[7, 8], with minor modifications. First, seawater was prepared by dissolving 38 g sea salt in 1 L of distilled water followed by filtration and transferring in a hatching container. Brine shrimp (*Artemia salina*) eggs were added to the one side of the divided tank where a lamp was placed and another side was covered. The shrimps were allowed for two days to hatch and mature as nauplii (larvae). The hatched shrimps were attracted to the lamp through the perforations in the dam and subjected to the bioassay. The ethanol extract of the plant were dissolved in dimethylsulphoxide (DMSO) and diluted with artificial sea water so that the final concentrations of extract were 10, 20, 40, 80 and 160 µg/mL and that of DMSO did not exceed 1%. 4 mL of the diluted samples or blank (DMSO and sea water) were taken in a series of vials. Each concentration was tested in triplicates. Then, 100 µL of suspension of nauplii containing 10 living shrimps was added into each vial and incubated for 24 h under the light of incandescent lamps. The vials were then examined and the number of dead nauplii in each vial was counted. After 10 min, 100 µL of methanol was added and the total numbers of dead nauplii in each well were counted to confirm the number of nauplii in each vial. The mortality percentage and LC_{50} (lethal concentration for 50% of the population) were determined using statistical analysis and the graph plotted concentration against percent lethality.

Phytochemical Analysis

The preliminary phytochemical studies were performed to detect the presence of different chemical groups in 10% (w/v) solution of the plant extract. Qualitative chemical tests for steroids, flavonoids, reducing sugars, tannins, gums, alkaloids and saponins were carried out using standard procedures [9,10].

Statistical Analysis

All data obtained were expressed as the mean ± standard error of mean (SEM). Statistical differences between the treatments and the controls were estimated by SPSS 11.5 software for Windows followed by the student's t-test. P values less than 0.05 was considered to be statistically significant.

Results and Conclusion

Diuretic test

Compared to control mice, those treated with plant extract showed marked increase in urine output during whole period of the experiment (Table 1). The extract caused significant increase in urine volume by 45 % ($P < 0.007$) and 91 % ($P < 0.0001$) in 1st and 2nd four hours of study, respectively. The diuretic index (total urine volume of treated group/total urine volume of control group) was found to be 1.32. The result suggests that the plant extract possesses significant diuretic effect in the experimental mice. The effect may be produced by stimulation of regional blood flow or initial vasodilation,[11] or by producing inhibition of tubular reabsorption of water and accompanying anions [12].

Brine shrimp bioassay

Ethanol extract of *D. trifoliata* showed different mortality rate of brine shrimp which increased proportionally with the increasing concentration of the extract (Fig. 1). From the graph, the concentrations of the crude extract at which 50% mortality (LC_{50}) and 90% mortality (LC_{90}) of brine shrimp nauplii occurred were found to be 10.00 µg/mL and 39.81 µg/mL, respectively.

The *in vivo* lethality test on a simple zoological organism, such as brine shrimp nauplii, has been used as a convenient tool for screening of bioactive natural products [7] Brine shrimp bioassay has good correlation with human solid cell lines [13]. A number of novel antitumer and pesticidal natural products have previously been isolated using this bioassay [7,13].

Therefore, the observed activity against the brine shrimp nauplii suggests that the crude extracts may contain antitumer, antibacterial or pesticidal compounds. It is possible that a broad range of structurally diverse compounds contribute to the overall pharmacological activity of the crude extract and synergistic effects between active principles may exist.

Furthermore, preliminary phytochemical analysis of ethanol and chloroform extracts of aerial parts of *D. trifoliata* revealed the presence of tannins, steroid, and flavonoids. There are some reports concerning diuretic properties[14] and cytotoxic activity of flavonoids[15]. So, flavonol glycosides might be active principles responsible for the observed biological activities.

In conclusion, the present study demonstrates that the ethanol extract of *D. trifoliata* contains pharmacologically active substance(s) possessing significant diuretic and toxicity activities. However, further studies will be necessary to isolate and characterize the active principles and to evaluate the mechanisms involved in the observed biological activities.

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Table 1: Effect of *D. trifoliata* extract on urination

Treatment	Urinary volume (mL) 3rd 4 hrs			
	1st 4 hrs	2nd 4 hrs	3rd 4 hrs	Last 11 hrs
Control	0.29 ± 0.03	0.46 ± 0.01	0.55 ± 0.03	2.00 ± 0.15
Extract (1000 mg/Kg)	0.42 ± 0.02*	0.88 ± 0.02**	0.75 ± 0.04	2.33 ± 0.19

Values are expressed as mean ± SEM (n = 6), *P < 0.007 vs control, **P < 0.0001 vs control, Student's t-test.

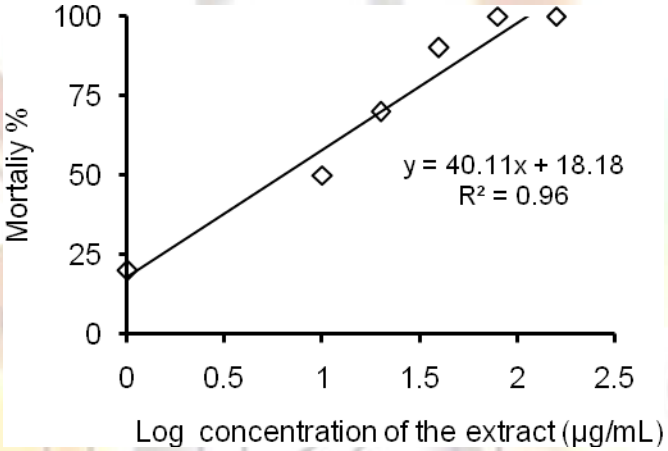

Fig. 1: Mortality rate (%) of brine shrimp (at 24 h) versus various concentration of *D. trifoliata* extract