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## PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIDIARRHOEAL ACTIVITY OF *DERRIS TRIFOLIATA* LOUR.

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### ABSTRACT

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*Derris trifoliata* Lour,  
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The present study was designed to investigate the antidiarrhoeal potential of 80% ethanol extract of aerial parts of *Derris trifoliata* (DT) on castor oil-induced diarrhoea in mice. Phytochemical screening of the plant extracts for their active constituents was also carried out using standard procedures. Oral administration of ethanol extract of DT (500 and 1000 mg/kg) significantly, and dose-dependently delayed the onset of diarrhoea induced by castor oil and also significantly reduced the number of diarrhoeal episodes and the number of animals exhibiting diarrhoea. The results were comparable to those of standard antimotility drug, hyoscine butylbromide (50 mg/kg). Phytochemical screening revealed the presence of steroid, flavonoid, reducing sugar, tannin, gum and saponin as major constituents. The results point out the presence of some active principles in DT extract possessing anti-diarrhoeal effect and substantiate the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine.

**INTRODUCTION:** Diarrhoea is a potential cause of morbidity and mortality especially in infants and children in the developing countries<sup>1</sup>. Medicinal plants are promising source of antidiarrhoeal drugs<sup>1, 2</sup>. For this reason, international organizations, such as WHO have encouraged studies for treatment and prevention of diarrhoeal diseases depending on traditional medicinal practices<sup>3</sup>. Currently, a number of medicinal plants with antidiarrhoeal and antimicrobial properties is used in traditional herbal practice in many countries of Asia including India and Bangladesh<sup>4, 5</sup>. So, it is important to identify and evaluate commonly available natural drugs that could be used against any type of diarrhoeal disease.

*Derris trifoliata* (DT) of the family Fabaceae, alternatively Leguminosae 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 evergreen shrub, reaching a length of 8 meters or less. Several rotenoids<sup>6, 7</sup> and glycosidic compounds<sup>8</sup> have been isolated from aerial parts of DT. Earlier report indicated a varied level of broad spectrum antimicrobial activity of extractives of *Derris trifoliata* and two other species of *Derris*<sup>9</sup>. Aerial parts of the plant have been traditionally used as a stimulant, antispasmodic and counter-irritant, and against diarrhoea and dysentery<sup>10</sup>.

However, these therapeutic potentials of the plant have not been scientifically evaluated. Therefore, the present study was undertaken to investigate scientifically the claimed biological activities of the aerial parts` extracts of DT against an experimental model of diarrhoea in mice.

## MATERIALS AND METHODS:

**Plant Material:** The plant DT was collected from the Koromjol Area of Sundarban 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 airtight 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) for assessing biological activity. The animals were kept in standard environmental conditions for at least one week for adaptation and had free access to standard laboratory food and water. All animal experiments were conducted on an isolated and noiseless condition in accordance with guidelines of the Animal Ethics Committee, Khulna University, Khulna, Bangladesh.

**Antidiarrhoeal Test:** The mice were screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the experiment. The test animals fastened for 24 h were randomly allocated to three groups consisting of six mice in each group. The animals of group I (control) received vehicles only (distilled water containing 0.1% Tween-80). Group-II (positive control) received standard antimotility drug, hyoscine butylbromide at a dose of 50 mg/kg body

weight as oral suspension. The group III (test groups) animals were treated with suspension of the extract of aerial parts of DT at an oral dose of 500 or 1000 mg/kg body weight. After one hour each animal was given 0.5 mL of castor oil by oro-gastric polyethylene catheter and placed in separate cages, the floor of which was lined with adsorbent paper. The characteristic diarrhoeal droppings were noted every hour in six hours study after the administration of castor oil<sup>11,12</sup>.

**Phytochemical Screening:** The extracts (10% w/v) of the plant were subjected to preliminary phytochemical screening with various qualitative chemical tests to identify the presence or absence of various classes of phytoconstituents. To perform the tests the following chemicals and reagents were used: reducing sugars with Fehling's test and Benedict's test, saponins with the capability of producing suds, steroids with Libermann-Burchard test and chloroform and sulphuric acid, flavonoids with Mg and HCl, tannins with ferric chloride test and Potassium dichromate test, gum with Molish reagents and sulfuric acid. Alkaloids were tested with Mayer's reagent, Hager's reagent and Dagendorff's reagent. These were identified by characteristic color changes using standard procedures<sup>13,14</sup>.

**Statistical Analysis:** All the data obtained were expressed as the mean  $\pm$  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:** Preliminary phytochemical screening of ethanol and chloroform extracts of aerial parts of DT revealed the presence steroid, flavonoids, reducing sugar, and gum as major active constituents (**Table 1**). However, saponin was absent in chloroform extract and alkaloids were absent in both extracts.

**TABLE 1: PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS OF DT**

Phytoconstituents	Ethanol extract	Chloroform extract
Steroid	+	+
Alkaloid	-	-
Reducing sugar	+	+
Gum	+	+
Saponin	+	-
Tannin	+	+
Flavonoid	+	+

"+" means presence and "-" means absence.

**Antidiarrhoeal Test:** In antidiarrhoeal experiment, castor oil (0.5 ml, p.o.) induced diarrhoea promptly within approximately one hour in all the animals and produced a considerable amount of stool. The ethanol extract of aerial parts of DT (500 and 1000 mg/kg) significantly prolonged the time for diarrhoeal induction by castor oil in a dose-dependent manner (**Table 2**). None of the plant extract treated animals showed diarrhoea up to at least one and half hour after administration of DT. Compared to control animals, the plant extract reduced significantly the number of diarrhoeal episodes. In an average, 64.6 % and 41.7 % of mice were protected against the diarrhoea by oral administration of the plant extract at the doses of 500 and 1000 mg/kg body weight, respectively.

The onset of castor oil-induced diarrhoea and the number of diarrhoeal episodes were also profoundly prolonged, and reduced, respectively by hyoscine butylbromide (50 mg/kg). The number of animals suffering from diarrhoea was also significantly reduced by the control drug by protecting 56.5 % of them against the diarrhea. The anti-diarrhoeal activity of the plant extract at a lower dose (500 mg/kg), was comparable to that of hyoscine butylbromide at a dose of 50 mg/kg body weight, especially at the middle stage of the total observation period. However, the anti-diarrhoeal activity of plant extract greatly improved at a higher dose (1000 mg/kg) throughout the full observation period.

**TABLE 2: ANTIDIARRHOEAL ACTIVITY OF ETHANOL EXTRACT OF DT AGAINST CASTOR OIL-INDUCED DIARRHOEA**

Treatment	Onset of diarrhoea (min)	Number of diarrhoeal episodes at time			
		1 h	2 h	3 h	4 h
Control	67.50± 7.03	3.16 ± 0.60	2.17 ± 0.48	2.34 ± 0.56	2.33 ± 0.94
HBB (50 mg/Kg)	116.05 ± 12.64*	0.67 ± 0.33	0.50 ± 0.34	0.83 ± 0.40	0.66 ± 0.21
Extract (500 mg/Kg)	113.33 ± 22.74**	1.16 ± 0.45	0.55 ± 0.33	0.88 ± 0.43	1.30 ± 0.50
Extract (1000 mg/Kg)	186.10 ± 14.05***	0.50 ± 0.12	0.16 ± 0.24	0.17 ± 0.18	0.33 ± 0.23

Values are expressed as mean ± SEM (n = 6), HBB = Hyoscine butylbromide. \*P < 0.005, \*\*P < 0.02, \*\*\*P < 0.001 vs control, Student's t-test.

**DISCUSSION AND CONCLUSION:** Diarrhoea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract accompanied with an excess loss of fluid in the faeces. Castor oil is hydrolyzed in the upper small intestine to ricinoleic acid that produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins<sup>15</sup>.

This condition induces an increase in the permeability of the mucosal cells and changes in electrolyte transport, which results in a hyper-secretory response (decreasing Na<sup>+</sup> and K<sup>+</sup> absorption and reducing Na<sup>+</sup>, K<sup>+</sup> ATPase activity in the small intestine and colon)<sup>16</sup>, stimulating peristaltic activity and diarrhea<sup>17</sup>.

The castor oil model therefore incorporates both secretory and motility diarrhea<sup>18</sup>. Inhibitors of prostaglandin synthesis are known to delay diarrhoea induced with castor oil<sup>19</sup>. The results of the present study show that the ethanol extract of DT significantly reduced the incident and severity of diarrhea. The plant showed significant anti-motility activity like the standard drug hyoscine butylbromide. These

observations suggest that the antidiarrhoeal effect of methanol extracts may be due to the inhibition of prostaglandin biosynthesis. The mechanism involved may be associated with dual effects on gastrointestinal motility as well as on water and electrolyte transport across the intestinal mucosa.

Furthermore, the standard chemical tests performed in this study showed that the leaves of the plant species contain tannins, saponins particularly steroidal saponin, and flavonoids. Tannins have been reported in several studies to have antidiarrhoeal effect<sup>20-22</sup>. In fact, tannins denature proteins and form protein tannate, which makes the intestinal mucosa more resistant and reduces intestinal secretion<sup>23</sup>.

The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion<sup>24-26</sup>. Hence, the antidiarrhoeal activity of the plant may be due to its content of tannins and/or flavonoids. In addition, the anti-diarrhoeal activity of DT may be associated with its antimicrobial effect<sup>9,27</sup>.

In conclusion, the present study demonstrates that the ethanol extract of DT contains pharmacologically active substance(s) possessing significant antidiarrhoeal activity. The present data provided a support for the traditional use of the plant as an antidiarrhoeal remedy. However, more detailed phytochemical analysis will be necessary to isolate and characterize the active compounds which are responsible for the antidiarrhoeal effect and to understand exact mechanisms of action of this activity.

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