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PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIDIARRHOEAL ACTIVITY OF *FICUS HISPIDA* LEAVES

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ABSTRACT: As a source of remedies, medicinal plants are widely used as alternative medicines for the treatment or prevention of many diseases. *Ficus hispida* is traditionally used for treating diarrhea, wounds, pain, inflammation, diabetes, fever and neurological disorders. To evaluate the qualitative phytochemical constituents and antidiarrhoeal activity of methanolic extract of *Ficus hispida* leaves the present study was designed. Phytochemical constituents and antidiarrhoeal activities were determined and assessed by various tests such as Molisch's test, Fehling test, Mayer's test, frothing test, $FeCl_3$ test, alkali test, Salkowski's test, Keller-killiani test and $CuSO_4$ test, castor oil and $MgSO_4$ induced diarrheal test. This extract figured the presence of carbohydrates, flavonoids, tannins, glycosides, triterpenoids, fat and fixed oils. Moreover, both doses of (200 mg/kg and 400 mg/kg) methanolic extract of *Ficus hispida* leaves significantly ($p<0.05$, *vs*. control) reduced the gastro-intestinal motility and inhibit the percentage of diarrhea in antidiarrhoeal models. But 400 mg/kg dose showed better antidiarrhoeal activity than 200 mg/kg dose compared to control in both antidiarrhoeal tests. The results indicate that *Ficus hispida* leaves may provide a potential source of antidiarrhoeal activities.

INTRODUCTION: *Ficus hispida* is a member of the Moraceae family. It is a medium but well distributed species of tropical fig tree or shrub that is coarsely hairy and dioeciously. It is generally known as Dumoor in Bangladesh. *Ficus hispida* [Family: Moraceae; English nme: Hairy fig; Botanical name: *Ficus hispida*; Local name: Dumor, kack dumur] is a medicinal tree, which can attain a height up to 10 meters.

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It is commonly a popular plant which is widely distributed throughout subcontinent from Bangladesh to India and Malaysia and is also found in Australia [1]. Usually, the leaves are opposite, leaf blade ovate, oblong or obovatye-oblong. They measure 10 - 25 cm × 5 - 10 cm, thickly papery. Secondary veins are 6-9 on each side of the mid vein. The petiole measure 1 - 4 cm long with short thick hairs. The fig appears axillary on normal leafy shoots, measuring 1.2 - 3 cm diameter with short scattered hairs. The male flowers are numerous near the apical pore; calyx lobes 3, thinly membranous; stamen single. The gall flowers are without calyx, style subapical, short and thick. The female flowers are also without calyx, the style is lateral and with hairs [2].

The plant generally contains ficushispimines A and B, ficushispidine, hispiloscine, *β*-amyrin acetate, N-triacontanyl acetate, ficusin A, lupeol acetate, and 10-keto- tetracosyl arachidate [3, 4, 5] which are revealed from recent publication. Astringent, antidysenteric, antipsoriasis, antianemic, and antihemorrhagic properties of whole plant (bark, fruit, root, and leaves) has already been demonstrated and reported elaborately [6, 7].

The roots and leaves are comprehended for their antidiarrhoeal [8], antidiabetic [9], antibacterial [10], hepatoprotective [11], antioxidant [12], and cardio-protective [13] properties. The fruit is edible and acts as a coolant and tonic. A mixture of honey and its juice is a good antihemorrhagic [14]. Diarrhea has long been recognized as one of the most important health problems in developing countries. In Bangladesh diarrhea is a principle cause of infant mortality and morbidity. Treatment of diarrhea is generally non specific and is usually aimed to reducing the discomfort and inconvenience of frequent bowel movements. To overcome the menace of diarrheal disease in developing countries the World health organization (WHO) has included a programme for the control of diarrhea, which involves the use of traditional herbal medicine [15].

As a regular folklore medicine *Ficus hispida* leaves is practiced in some regions of Bangladesh and used against diarrhea, neurological disorders, pain, diabetes, fever and inflammation. So, medicinal compounds that derived from plant sources such as tannins, flavonoids, terpinoids, glycosides and coumarins could provide an excellent fountainhead to develop new antidiarrhoeal agent, which could be more efficacious, affordable, safer and accessible for patients. Therefore, the present study was designed to identify the phytoconstituents and justify the antidiarrhoeal activity of *Ficus hispida* leaves, and evaluate the traditional usage scientifically.

MATERIAL AND METHOD:

Collection and Identification of the Plant: For performed this study, green and freshness leaves of *Ficus hispida* plant was collected from Jessore University of Science and Technology Campus, Jessore, Bangladesh, in September, 2017. The collected leaves were identified and confirmed by National Herbarium, Bangladesh.

Extraction of Leaves: Around 250 gm of powdered leaves were taken for methanol extraction. First, the leaves of *Ficus hispida* were thoroughly washed with fresh water to remove all dirt and contaminants and dried in shade at room temperature (25 ± 2 °C) for two weeks. The materials were grinded into coarse powder and cold extraction method was used to extract the active components. The ground leaves (250 gm) were soaked in sufficient amount of methanol for 14 days at room temperature with periodical shaking and stirring. The whole mixture was primarily filtered through cotton and then through Whatman no. 1 filters. The solvent was evaporated with a rotary evaporator under reduced pressure at 40 °C temperature to yield semisolid crude extract. The percentage yield of the extract was 3.73% (w/w). The extract was then preserved in a refrigerator till further use.

Experimental Animals: For conducted the antidiarrhoeal study, fifty Swiss albino mice of either sex, aged 4 - 5 weeks, weighing about 25 - 30 gm were collected from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Before initiating the experiment, the animals were exposed to alternative 12:12 h light and dark cycle at an ambient temperature of 26 ± 2°C. Proper supplies of foods and water *ad libitum* were ensured. Institutional Animal Ethical Committee of Jessore University of Science and Technology, Jessore, Bangladesh was approved all protocols for this animal experiment. Mice were acclimatized for 7 days in the laboratory environment prior to the study, and maintained the constant environmental and adequate nutritional conditions throughout the period of the experiment.

Standard Drug: Loperamide HCl (purchased from ACME Laboratories Ltd., Bangladesh) was used as standard drug and was administered orally.

Phytochemical Screening: Freshly prepared *Ficus hispida* leaves extract were subjected to different qualitative tests.

Molisch's Test for Carbohydrates: Approxi-mately 500 mg of crude extract was dissolved in 5 mL of distilled water and later filtered. A few drops of Molisch's reagent (α-naphthol 10% (w/v) in 90% ethanol) were added to the filtrate. Then 1 mL

of concentrated H_2SO_4 was poured carefully along the side of the test tube. Two minutes later, 5 mL of distilled water was added. A positive test, indicating the presence of carbohydrates, was confirmed with formation of dull violet or red color at the interphase of the two layers [16].

Fehling's Test for Reducing Sugars: 2 mg plant extract was dissolved in 1 mL of distilled water and filtered. Then, 1 mL mixture of Fehling's solutions A and B (a ratio of 1:1) was added to the filtrate, which was heated in a water bath for a few minutes. Formation of brick-red precipitate confirmed the presence of reducing sugars [17].

Mayer's Test for Alkaloids: In Mayer's test, one or two drops of 0.35 mol/l Mayer's reagent (potassium - mercuric iodide solution, 1.36 g mercuric chloride and 5 g of potassium iodide, dissolved in 100 ml distilled H_2O) was added to 2 mL (50 mg extract dissolved in 5 ml of 1% aqueous HCl) filtrate along the side of the test tube. A positive test, demonstrating the presence of alkaloids, was indicated by a white creamy precipitate [18].

Frothing Test for Saponins: 100 mg plant extract was dissolved in 10 ml of methanol for making stock solutions. These stock solutions were diluted to 0.5 mg/ml by the additions of 20 ml of distilled water. Test tube containing the dilution was then shaken for 15 min. Formation of foam on the top of the test tubes indicated the presence of saponin [17].

$FeCl_3$ Test for Tannins: 50 mg plant extract was dissolved in 5 ml distilled water, followed by the addition of a few drops of 5% $FeCl_3$. Tannin was confirmed by the development of a bluish- black color [19].

Alkali Test for Flavonoids: For this test, a few drops of 5% NaOH solution were added to 1 ml of filtered stock solution (100 mg of extract dissolved in 10 ml of methanol), which produced a deep-yellow color. The color was lost in the presence of dilute HCl and confirmed flavonoids [19].

Salkowski's Test for Triterpenoids: 2 mg plant extract was shaken in 1 ml of $CHCl_3$. Then, a few drops of concentrated H_2SO_4 were added to the solution along the side of the test tube. Development of a red-brown color at the interface indicated the presence of triterpenoids [17].

Keller-killiani Test for Glycosides: For this screening, 1 ml of extract, 1 ml of glacial acetic acid and few drops of 2% $FeCl_3$ were added and then 1 ml of conc. H_2SO_4 is also added in the mixture. Appearance of Brown ring shows presence of glycosides [20].

$CuSO_4$ Test for Fat and Fixed Oils: 5 drops of extract solution (0.25 g extract dissolved in 25 mL mother solvent) mixed with 1 ml of 1% $CuSO_4$ and then few drops of 10% NaOH was added. Appearance of clean blue solution shows presence of fat and fixed oils.

Antidiarrhoeal Study:

Castor Oil Induced Antidiarrhoeal Test: Diarrhea was induced in mice using a slight modified method of Shoba and Thomas [21]. By administering 0.5 ml of castor oil orally the preliminary screening of animals were performed, and those animals that started diarrhea were selected finally for the test. Twenty diarrheal screened mice were divided into control group (distilled water), positive control or standard group (loperamide HCl, 3 mg/kg b.w.), and test groups (MFHL 200 mg/kg and 400 mg/kg b.w.), containing five mice in each group. Experimented animals were fasted for around 16 h with water *ad libitum*. Mice in the control group, standard group and test groups orally received one dose of distilled water, loperamide HCl, MFHL 200 mg/kg and 400 mg/kg respectively. Then, each animal received 0.5mL of castor oil orally for initiating diarrhea after 30 min of the above treatments. Observation for defecation continued up to 4 h on blotting paper lined individual cage was used for placing every animal. These papers were replaced every hour. The number of diarrheal feces was count and recorded for a period of 4 h and the percentage of inhibition of defecation was calculated for every group of animals.

$MgSO_4$ Induced Antidiarrhoeal Test: The method described by Doherty [22] was applied for this study with slight modification. Here, a similar procedure as for castor oil induced diarrhea test was maintained for magnesium sulphate induced diarrheal model.

The animals all were screened for diarrhea was done by administering magnesium sulphate at a dose of 2 g/kg orally. Experimented animals were fasted for 16 h with water *ad libitum*. Then, mice were grouped and treated as described before. Then, each animal received 2g/kg of magnesium sulphate orally for initiating diarrhea after 30 min of the above treatments. Observation for defecation is same as for castor oil induced diarrhea test, and the antidiarrhoeal activity was expressed by comparing the percent of inhibition of defecation of different groups with control group.

Statistical Analysis: The experimental results were expressed as mean ± SEM (standard error of mean). Statistical analyses for antidiarrhoeal studies were evaluated by one-way ANOVA following Dunnett's test through the SPSS software (version 16; IBM Corporation, New York, USA). The obtained results were compared with the vehicle control group. The $p<0.05$ was considered to be statistically significant.

RESULTS:

Phytochemical Screening: After evaluation the pharmacological activities of plant extract, it is important to depict the chemical nature of plant materials. Phytochemical screening of the *Ficus hispida* leaf showed the presence of several primary and secondary metabolites, or phyto-constituents, which are summarized in **Table 1**. In the phytochemical screening, MFHL showed the presence of almost all of the phytoconstituents like carbohydrates, flavonoids, tannins, phenols, glycosides, triterpinoids, fat and fixed oils that were tested here. However, some tests did not show consistent results such as carbohydrate content in MFHL was indicated by Molisch's test, but not by Fehling's test.

TABLE 1: PHTOCHEMICAL SCREENING OF METHA-NOLIC EXTRACT OF *FICUS HISPIDA* LEAVES

Phytoconstituents	Test name	Observation
Carbohydrates	Molisch's test	+
	Fehling's test	-
Alkaloids	Mayer's test	-
Saponins	Frothing test	-
Tannins	$FeCl_3$ test	+
Flavonoids	Alkali test	+
Triterpenoids	Salkowski's test	+
Glycosides	Keller-killiani test	+
Fat and Fixed oils	$CuSO_4$ test	+

'+' mean presence of specific phytoconstituents and '-' mean absence of specific phytoconstituents

Antidiarrhoeal Study:

Castor Oil Induced Antidiarrhoeal Test: In the castor oil induced diarrheal mice, loperamide HCl (3 mg/kg) and methanolic extract of *Ficus hispida* leaves at the doses of 200 mg/kg and 400 mg/kg significantly ($p<0.05$, *vs*. control) reduced the total number diarrheal feces. Here, the decrease of the total number of diarrheal feces is dose dependent in manner. Highest and significant ($p<0.05$, versus control) percentage of inhibition of diarrhea (61.11%) was revealed by MFHL 400 mg/kg. Castor oil induced antidiarrhoeal results are showed in **Table 2**.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF *FICUS HISPIDA* LEAVES IN CASTOR-OIL INDUCED DIARRHEAL TEST

Group	Dose	Number of diarrheal feces	% of inhibition of diarrhea
Control	10 mL/kg	7.20±0.86	-
Loperamide HCl	3 mg/kg	1.60±0.24	77.78
MFHL	200 mg/kg	4.20±0.58	41.67
MFHL	400 mg/kg	2.80±0.37	61.11

Numbers of feces are presented as mean ± SEM (standard error of mean). P<0.05, *vs* control (Dennett's t test)

$MgSO_4$ Induced Antidiarrhoeal Test: In the magnesium sulphate induced diarrheal mice, loperamide HCl (3 mg/kg) and methanolic extract of *Ficus hispida* leaves at the doses of 200 mg/kg and 400 mg/kg significantly ($p<0.05$, *vs*. control) reduced the total number diarrheal feces. Here, the decrease of the total number of diarrheal feces is dose dependent in manner.

Highest and significant ($p<0.05$, versus control) percentage of inhibition of diarrhea (58.06%) was revealed by MFHL 400 mg/kg. Magnesium sulphate induced antidiarrhoeal results are showed in **Table 3**.

TABLE 3: EFFECT OF METHANOLIC EXTRACT OF *FICUS HISPIDA* LEAVES IN $MgSO_4$ INDUCED DIARRHEAL TEST

Group	Dose	Number of diarrheal feces	% of inhibition of diarrhea
Control	10 mL/kg	6.20±0.58	-
Loperamide HCl	3 mg/kg	1.20±0.37	80.65
MFHL	200 mg/kg	3.60±0.60	41.77
MFHL	400 mg/kg	2.60±0.40	58.06

Numbers of feces are presented as mean ± SEM (standard error of mean). P<0.05, *vs* control (Dennett's t test)

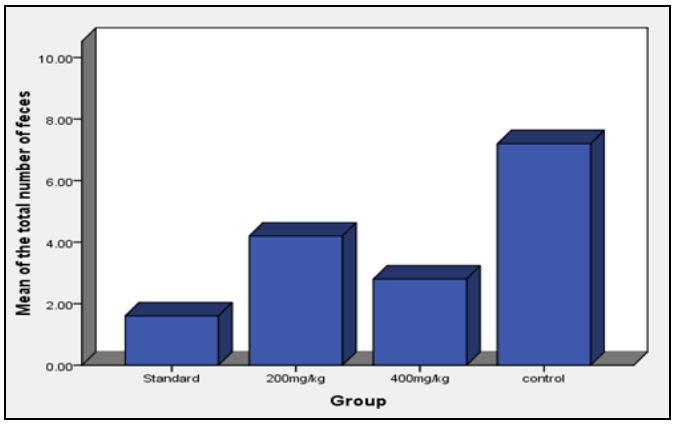

FIG. 1: EFFECT OF METHANOLIC EXTRACT OF *FICUS HISPIDA* LEAVES IN CASTOR-OIL INDUCED DIARRHEAL TEST

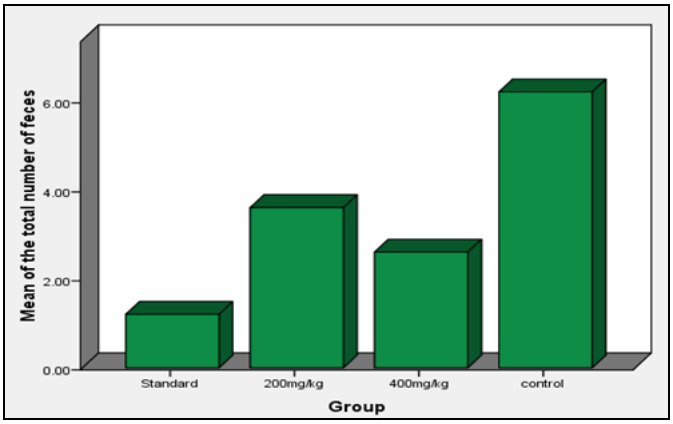

FIG. 2: EFFECT OF METHANOLIC EXTRACT OF *FICUS HISPIDA* LEAVES IN $MgSO_4$ INDUCED DIARRHEAL TEST

X-axis – group of experimented animal; Y-axis – number of diarrheal feces
Level of Significance = $p<0.05$ compared to control (Dennett's t test)

DISCUSSION: The phytochemical analysis of the leaves of *Ficus hispida* revealed the presence of carbohydrates, tannins, flavonoids, glycosides and triterpenoids which are act as palliative of pain, inflammation, diarrhea, fever, neuropharmacological disorders and diabetes.

Diarrhea (loose motions) is a condition in which feces are discharged from the bowels frequently and in a liquid form. It may be characterized as the abnormally frequent expulsion of feces of low consistency which may be due to a disturbance in the transport of water and electrolytes in the intestines [23]. Prostaglandins strongly contribute to the pathophysiological functions in the gastro-intestinal tract. The major cause of arachidonic acid-induced diarrhea is release of prostaglandins [24]. It also characterized by an increase in secretion of water and electrolytes, as well as an increase in intestinal transit and an increase in watery feces (diarrheal feces).

Traditionally, prevalence of diarrhea can be controlled by using *Ficus hispida*. Castor oil (diarrheal agent) increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water, which is associated with prostaglandin release [25]. As a result, absorption of sodium and potassium ions are reduced, which sequentially lessens the function of Na^+, K^+ -ATPase in colon plus small intestine [26]. In the study, both doses of methanolic extract (200 mg/kg and 400 mg/kg) of *Ficus hispida* leaves showed a significant inhibition ($p< 0.05$, versus control) of castor oil induced diarrhea in mice and may be due to the inhibition of electrolyte permeability of the intestine and prostaglandin release.

In another experiment, after the oral administration of magnesium sulphate, results the gathering of fluid in the intestinal lumen and its movement from proximal to the distal intestine occurs. Discharge of cholecystokinin and nitric oxide from duodenal mucosa occurs after its oral administration. Then two recurrently results come about and one is the inhibition of reabsorption of NaCl and water that occurs from the previous case. Another is the rise of secretion and motility of small intestine [27]. Methanolic extract of *Ficus hispida* leaves extract (200 mg/kg and 400 mg/kg) was effective in reducing diarrhea and that was expected due to increase in electrolyte and water reabsorption from the gastrointestinal tract.

Above activities are seems to be due to the presence of tannins in the methanolic extract of *Ficus hispida* leaves. In fact tannins are responsible for the denaturation of proteins and form protein tannate, which reduces the intestinal mucosa permeability [28]. The methanolic extract of *Ficus hispida* leaves was administered at the dose of 200 mg/kg and 400 mg/kg showed 41.67% and 61.11% reduction of diarrhea in castor-oil induced diarrheal test and 41.47% and 58.06% reduction of diarrhea in $MgSO_4$ induced diarrheal test respectively. So we can conclude that the present study seems to support the claims of a traditional medicine practitioner about the use of *Ficus hispida* in diarrhea.

CONCLUSION: From the results of existing study, it can be concluded that methanolic extract of *Ficus hispida* leaves might possess remarkable antidiarrhoeal properties. Data obtained from this study showed that all activities were dose dependent in manner and statistically significant. It is also reasonable to believe that the methanolic extract of *Ficus hispida* leaves might be effective in inflammatory diarrhea, secretary diarrhea and infectious diarrhea.

The presence of tannins, flavonoides, *β*-amyrin acetate, lupeol acetate, and phenolic compounds might be responsible for these activities. On the basis of these findings we hope that, further detailed investigation is needed to confirm to find out the active components of the extracts for discovering the mechanism of actions in the improvement of antidiarrhoeal agents. Moreover, it could be potential source for novel 'lead' discovery for antidiarrhoeal drug development.

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CONFLICT OF INTEREST: The authors declare that there is no conflict of interests regarding the publication of this paper.

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