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Research Article

**ANTIBACTERIAL, ANTIOXIDANT AND CYTOTOXIC
ACTIVITIES OF *FICUS HISPIDA* LEAVES AND FRUITS**Dewan Md. Sumsuzzman³, Turabe Farah Chowdhury⁴, Israt Jahan Bulbul¹, Yesmin Begum^{2*}¹Assistant Professor, Department of Pharmacy, Southeast University, Banani, Dhaka -1213.^{2*}Assistant Professor, Department of Pharmacy, Southeast University, Banani, Dhaka -1213.³Department of Pharmacy, Southeast University, Banani, Dhaka – 1213.⁴Department of Pharmacy, Southeast University, Banani, Dhaka – 1213.**Abstract:**

The aim of conducting this study is to investigate the antibacterial, antioxidant and cytotoxic activities of the ethanolic leaf and fruit extracts of *Ficus hispida* to rationalize its primitive medicinal contemplation. *F. hispida* was subjected to bacterial inquisition by disc diffusion stratagem in opposition to Gram positive and Gram negative bacteria conducting kanamycin as standard. The leaf extract ascertained good antibacterial activity against *Pseudomonas aeruginosa* with 13 mm zone of inhibition and moderate activity against *Bacillus cereus* and *Vibrio parahaemolyticus* with 11mm and 10 mm zone of inhibition respectively. But the organisms were not sensitive to the fruit extract. Antioxidant activity of the plant was evaluated spectrophotometrically using DPPH and ascorbic acid as standard. The leaf and fruit extracts explicated significant antioxidant activity with the IC50 value of 41.68 µg/ml, 28.39 µg/ml successively compared with the standard ascorbic acid at 45.78 µg/ml. The cytotoxic activity of *F. hispida* was scrutinized by brine shrimp lethality bioassay utilizing vincristine sulfate as standard. The ethanolic leaves and fruits extract revealed auspicious cytotoxic activity with LC50 value of 10.32 µg/ml, 14.69µg/ml consecutively, in where; LC50 of Vincristine sulphate was 6.11 µg/ml. This article may provide optimistic empirical data to fact-finders and it can be further outstretched to disengage the bio-active components owing to determine the antioxidant as well as cytotoxic activity in human cell line.

Key words: *Ficus hispida*, Antibacterial, Antioxidant, Cytotoxic activity.**Corresponding Author:****Yesmin Begum,**

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INTRODUCTION:

F. hispida (Family: Moraceae) is a moderate sized tree and distributes throughout Bangladesh, India, Sri Lanka, Myanmar, southern region of China and Australia. Utmost parts of this plant are utilized as a folklore remedy for the treatment of various ailments by the Indian traditional healers, surprisingly the leaves are of particular interest from a medicinal point of view as an antidiarrheal, hepatoprotective, anti-inflammatory, antitussive, antipyretic, astringent, vulnerary, hemostatic and anti-ulcer drug, among the other parts [1-5]. Entire parts of this plant are found to be acrid, astringent, bitter, coolant and have activity against dysentery, ulcers, biliousness, psoriasis, anemia, piles and jaundice. Additionally, the fruit is known to be active as aphrodisiac, tonic, lactagogue and emetic [6-8]. 6-O-methyltylophorinidine and 2-demethoxytylophorine, and a novel biphenylhexahydroindolizinehispidine was isolated from stem and leaves of *F. hispida* [9]. Recently, hispidin has been reported to have anticancer activity [10]. Moreover 6-O-methyltylophorinidine which has been reported from this plant to exhibit cytotoxicity for lung, colon, nasopharynx and prostate cancer cell lines [11]. The phytochemical studies indicated the presence of triterpenoids and flavonoid in the methanolic extract of leaves of *F. hispida* having antioxidant activity, [12,13] these activities may be helpful in the holistic treatment of Alzheimer's disease and other age-related memory impairments [14]. Even through its significant traditional medicinal worth, this plant has not been investigated extensively till now with respect to pharmacognostical, traditional use, phytochemical and therapeutic parameters.

MATERIALS AND METHODS:

Drugs and Chemicals

DPPH (1, 1-diphenyl - 2-picryl hydrazyl) was obtained from Sigma Aldrich USA. Ascorbic acid was obtained from SD Fine Chem. Ltd, Biosar, India. DMSO (dimethylsulfoxide) was purchased from Merck, Germany. Kanamycin was collected from Square Pharmaceuticals Ltd., Bangladesh. Vincristine sulfate was collected from Alfa Aesar Ltd. USA.

Instrumentation

The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts using UV-spectrophotometer (Model NO. 1501PC Shimadzu, Japan) at 517 nm.

Collection and identification of the plant

The fresh leaves and fruits of *F. hispida* were collected in June 2014 from Brahmanbaria district, Bangladesh and authenticated at Bangladesh

National Herbarium, where a voucher specimen No. DACB 35853 has been deposited.

Extraction of the plant material

The collected fresh leaves and fruits were sun dried for seven to twelve days. The dried plant parts were ground into small powder by a grinder machine. Then 50 gm of powder of leaves and fruits were extracted separately by cold extraction process using ethanol (300 ml) with daily shaking and stirring for 7 days at room temperature. After 7 days the extracts were filtered through cotton followed by filter paper (Double filter paper 102, 11.0 cm). Then the liquid extracts were dried at room temperature (37 °C) to obtain a greenish mass.

Microbial strains and culture media

Antimicrobial activity was carried out against seven Gram negative bacteria such as *Vibrio parahemolyticus*, *Vibrio mimicus*, *Shigella dysenteriae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella paratyphi* and five Gram positive bacteria such as *Staphylococcus aureus*, *Sarcina lutea*, *Bacillus megaterium*, *Bacillus cereus* and *Bacillus subtilis*. These bacteria were chosen to be studied as they are important pathogens and also due to rapidly developed antibiotic resistance. The microorganisms were collected as pure cultures from the Institute of Nutrition and Food Science (INFS), University of Dhaka, Bangladesh. For bacteria, the culture media was prepared by nutrient agar, reconstituting with distilled water according to specification (2.8% w/v).

Antibacterial screening by disc diffusion method

Antibacterial activities of leave and fruit extracts of *F. hispida* were carried out *in vitro* by the standard disc diffusion method [15]. In this method, solutions of known concentration (500µg /disc) of the test samples were made by dissolving measured amount of the samples (50 mg) in 1 ml of methanol. Then sterile filter paper discs (5 mm diameters) were impregnated with known test substances and dried. The dried discs were placed on plates (Petri dishes, 120 mm diameters) containing a suitable medium (nutrient agar) seeded with the test organisms. Standard disc of kanamycin (30µg/disc) and blank discs (impregnated with solvents followed by evaporation) were used as positive and negative control. These plates were kept at low temperature (4 °C) for 24 hours to allow maximum diffusion. The plates were then kept in an incubator (37 °C) for 24 hours to allow the growth of microorganisms. Antibacterial activity of the test samples was observed by growth inhibition of organisms forming clear, distinct zone surrounding the discs. The antibacterial activity was expressed

in terms of millimeter by measuring the diameter of the zone of inhibition. The greater zone of inhibition indicates the greater activity of the test material against the test organism.

Cytotoxicity screening by brine shrimp lethality bioassay

The brine shrimp lethality bioassay was used to predict the cytotoxic activity [16, 17] of the extracts of *F. hispida*. The eggs of brine shrimp (*Artemia salina*) were hatched in a tank in artificial seawater (3.8% NaCl solution) at a temperature around 37⁰ C with constant air supply. For the experiment, the samples are prepared by dissolving the extracts in dimethylsulfoxide (DMSO) not more than 50µl in 5 ml solution and solutions of varying concentrations (20, 40, 60, 80 and 100µg/ml) were prepared by the serial dilution process using simulated seawater and a vial containing 50µl DMSO diluted to 5ml was used as a control. Then 10 live brine shrimp nauplii were added to each of the experimental vial. After 24 h, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. Vincristine sulfate was used as positive control. From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated.

Antioxidant activity by DPPH radical scavenging activity

The free radical scavenging activity (antioxidant capacity) of the plant extracts on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand-Williams [18]. During this experiment the test samples of the extracts of *F. hispida* at different concentrations were mixed with 3.0 ml of DPPH methanol solution. The antioxidant potentiality was assayed from the bleaching of purple colored methanol solution of DPPH radical by the plant extracts by UV- spectrophotometer at 517 nm. Ascorbic acid was used as a positive control. Percent scavenging of the DPPH free radical was measured using the following equation-

$$\% \text{ DPPH radical scavenging} = [1 - (\text{As}/\text{Ac})] \times 100$$

Here,

Ac = absorbance of control,

As = absorbance of sample solution.

Then % inhibitions were plotted against respective concentrations used and from the graph IC50 was calculated. The lower IC50 indicates higher radical scavenging activity and vice versa.

RESULTS AND DISCUSSION:

The leaf and fruit extracts of *F. hispida* were screened against Gram positive and Gram negative bacteria. Few organisms were found to be responsive to the extracts which were shown in (Table 1). Among the two extracts leaf exhibited good activity against *P. aeruginosa* with 13 mm zone of inhibition, against *B. cereus* and *V. parahaemolyticus* with 11 and 10 mm zone of inhibition respectively. The fruit extract revealed poor activity with average zone of inhibition of (5.0 mm -8.5 mm) and maximal zone of inhibition against *B. subtilis* with 8.5 mm. In imitation of the procedure of Meyer, the lethality of the extracts of *F. hispida* to brine shrimp was discerned and the results (% mortality at different concentrations and LC50 values) were shown in Fig. 1. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality [19] was plotted on the graph paper and the values of LC50 were calculated using Microsoft Excel 2007. The percent mortality increased with an increase in concentration. The leaf extract showed potential cytotoxic activity having an LC50 value of 10.32µg/ml in where LC50 value of standard vincristine sulfate at 6.11µg/ml. And the fruit extract revealed significant cytotoxic activity having an LC50 value of 14.69µg/ml. So, this plant. The antioxidant activity of the extracts was evaluated by using DPPH. The ethanolic leaf and fruit extracts showed in antioxidant activity with the IC50 value of 41.68 µg/ml, 28.39 µg/ml respectively compared with the standard ascorbic acid at 45.78 µg/ml. (Fig. 2).

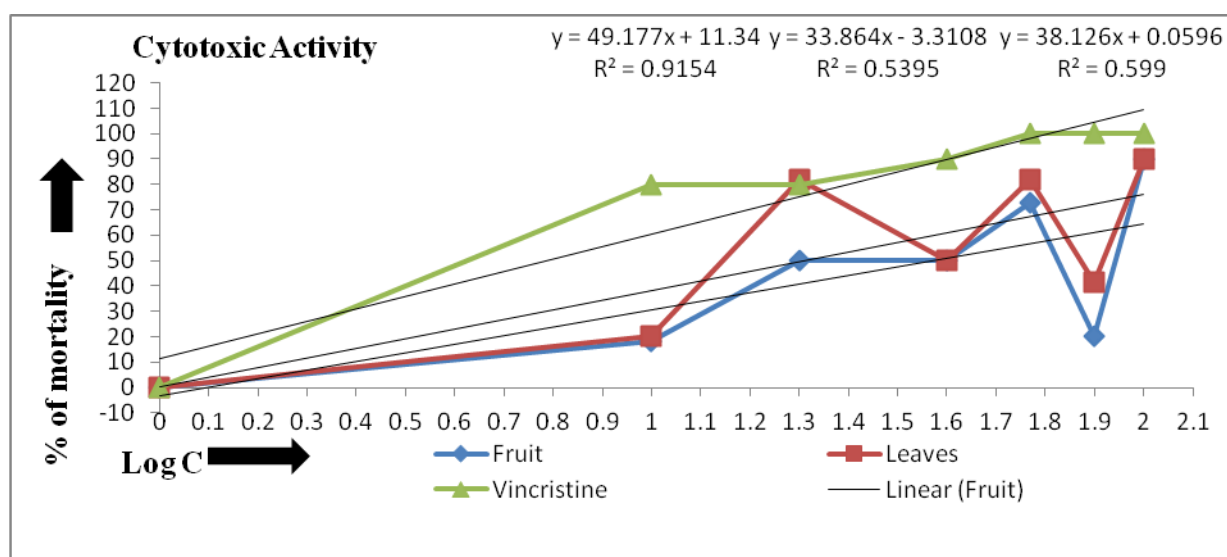
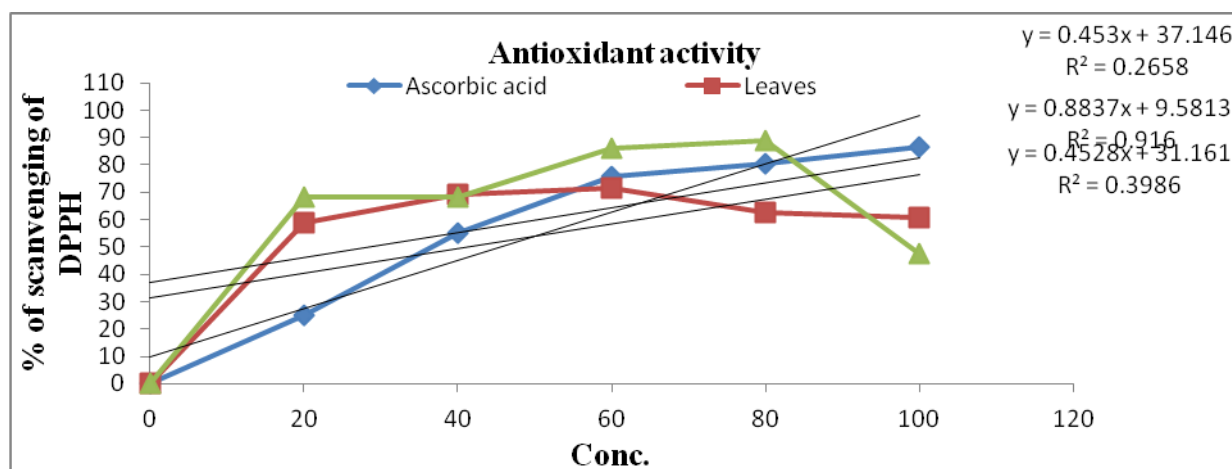
The ethanolic leaf and fruit extracts of *F. hispida* possess potential antioxidant and cytotoxic activities that verifies its folklore and traditional uses worldwide.

CONCLUSION:

Plants are the significant sources of potent compounds for the furtherance of novel natural therapeutic agents. Both the traditional literatures and research article recommend that *F. hispida*, a plant with predominant medicinal value which are formerly investigated for the treatment cancer, tumor etc. The outcome of this initiatory evaluation confers strong evidence that two parts of *F. hispida* could be considered as potential resources of antioxidant and cytotoxic drug constituents. Inspire with whole hearted efforts placed at the time of work, it is felt that there are lots of scope for further exploration are essential in order to isolate the active compound to evaluate the antioxidant as well as cytotoxicity activity in human cell line.

Table 1: Antibacterial Screening of different parts of *F. hispida*.

Group	Test Organisms	Diameter of Zone of Inhibition (mm)		
		Leave 500µg/disc	Fruits 500µg/disc	Kanamycin 30µg/disc
Gram positive bacteria	<i>B. subtilis</i>	9.5	8.5	32
	<i>S. lutea</i>	9.5	6.5	29
	<i>B. cereus</i>	11	7	27
	<i>S. aureus</i>	9.5	6.5	26
	<i>B. megaterium</i>	-	-	33
Gram negative bacteria	<i>S. paratyphi</i>	-	-	31
	<i>V. parahaemolyticus</i>	10	-	30
	<i>V. mimicus</i>	9	7	32
	<i>S. dysenteriae</i>	-	-	32
	<i>E. coli</i>	-	-	31
	<i>P. aeruginosa</i>	13	5	30

**Fig 1: Determination of LC50 values for standard and ethanolic extracts of leaves and fruits of *F. hispida* from linear correlation between logarithms of concentration versus percentage of mortality.****Fig 2: Determination of IC50 values for standard and ethanolic extract of leaves and fruits of *F. hispida* from linear correlation between of concentration versus percentage of scavenging of DPPH.**

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