ORIGINAL ARTICLE

Evaluation of Protein Denaturation, Membrane Stabilizing and Cytotoxic Activities of Ethanol Extract of *Ficus hispida* L. Fruits

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Abstract

The present study was conducted to investigate the protein denaturation, membrane stabilizing and cytotoxic activities of ethanol extract of Ficus hispida L fruits. Preliminary phytochemical screenings of the crude extractives demonstrated the presence of carbohydrates, glycosides, tannins, terpenoids, saponins, flavonoids, and steroids. In protein denaturation test, the extracts inhibited protein denaturation by 9.56% at 600µg/ml, 5.03% at 300µg/ml and by 2.00% at 150µg/ml. The membrane stabilizing activity was determined using hypotonic solution induced haemolysis of human red blood cells. In the membrane stabilizing assay, the crude ethanol extract exhibited inhibition of haemolysis of human RBCs by 29.59% at 600µg/ml, 17.16% at 300µg/ml and by 8.28% at 150µg/ml. The brine shrimp lethality bioassay is a rapid and widely used method that indicates cytotoxicity, anticancer, antiviral, pesticidal, insecticidal activities of the bioactive metabolites. In the cytotoxicity screening, the ethanol extract showed that the degree of lethality was directly proportional to the concentration (LC50 165.96µg/ml) of the crude extract.

Key words: ***Ficus hispida L. fruits, Protein denaturation, Membrane stabilizing, cytotoxic activity, Ethanol extract.***

1. Introduction

Natural products have played a vital role in the treatment of human diseases because they contain different chemical components of therapeutic values. Natural products have come from different sources namely plants, microorganisms, marine organisms, terrestrial vertebrates, invertebrates etc. [1, 2]. Among these, plants are the most valuable source for producing active compounds [3]. World Health Organization (WHO) reported that, more than 80% of the people depend on plants derived medicine for their primary healthcare needs [4]. A number of modern important drugs have been derived from plants used as antibiotics, antimalarial, anticancer, antidiabetic, antihypertensive drugs etc. [5].

Plants found in Bangladesh having different nutritional and phytochemical properties [6-8]. Carbohydrates, proteins, minerals, vitamins etc are the most important nutrients found in plants [9]. The plants also contain numerous biochemical constituents of medical interest including alkaloids, glycosides, tannins, resins, saponins, terpenes, phenolic compounds etc. In this case, Moraceae family may be good source of medicinally active compounds.

Many species from the Moraceae family have been used for their medicinal properties. Among the different species of *Ficus, Ficus hispida* L. (Family: Moraceae) locally known as dumoor in Bangladesh is an important fruit due to its different pharmacological activities. It grows in evergreen forest having moderate height, found in moist areas, near banks of many stream. It is mainly cultivated in India, Andaman Island, Myanmar, and Srilanka [10]. Fruit acts as a coolant and tonic. The fruit juice uses as a mild purgative. A mixture of honey and its juice has good antihaemorrhagic activities [11]. It is also used in the treatment of arthritis and different types of inflammation.

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Inflammation is a complex biological response of body tissues associated with swelling that occurs by injury or an infection [12]. It is a crucial aspect of host response that maintains healthy state against bacterial and viral infections [13], but an uncontrolled and aberrant inflammation may lead to many acute and chronic diseases [14]. To prevent inflammation, there is an emergency need to find out new anti-inflammatory drugs. Many new drugs have been discovered over the last few decades, but the treatment of inflammatory diseases is still a challenge. Non-steroidal anti-inflammatory drugs were used as first drugs to treat inflammatory diseases [15], but these drugs possess various side effects including heart diseases [16, 17], bleeding, nausea, vomiting, dyspepsia, gastric ulceration, diarrhea etc. [18]. In this respect plant extracts are in major focus.

Preliminary phytochemical screening of *Ficus hispida* L. fruits showed the presence of carbohydrates, glycosides, tannins, terpenoids, saponins, flavonoids and steroids. Therefore, the ethanol fruit extract of *F. hispida* L. could be potential source for future development of anti-inflammatory, membrane stabilizing and anticancer drugs with fewer side effects.

2. Materials and methods

2.1 Collection and identification of Plant

In this study, *Ficus hispida* L. fruits were collected from Sirajgonj, Bangladesh in the month of October 2016, and were identified by the experts at the Department of Botany, University of Rajshahi, Bangladesh.

2.2 Preparation and Extraction

After collecting the fruits, the extraneous and undesired substances from the plant material were removed by washing with water and the fruits were then dried at 50°C temperature in hot air oven [19]. After grinding, the powdered materials were stored in airtight containers and kept in a cool and dry place. About 200 gm of powered material was taken in a clean glass container and soaked in 800 ml of ethanol at room temperature for 10 days. Whatman filter paper (size 1) was used to filter the extracts and the filtrates were concentrated with a rotary evaporator using reduced temperature and pressure. Then the concentrated ethanol extracts was collected and stored in the refrigerator for further use.

2.3 Drug and Reagents

Diclofenac sodium was collected from Drug International Ltd., Dhaka, Bangladesh. All chemicals and reagents were at analytical grade.

2.4 Phytochemical Screening of crude extracts

The crude extracts were tested for the presence of chemical constituents, by using the following reagents viz carbohydrates were identified by the Fehling's solution and Benedict test, glycosidess with Legal's test, terpenes with the use of copper acetate, tannins with ferric chloride, saponins with froth and foam test, flavonoids with lead acetate test and steroids with Salkowski and Libermann-Burehared test [20-22].

2.5 Protein denaturation activity

Tissue proteins denaturation is one of the main causes of inflammatory and arthritic diseases. Due to proteins denaturation, auto antigens are produced in our body that cause arthritic diseases. The extracts that can prevent protein denaturation lead to the development of anti-inflammatory drugs. Therefore, in this study, the in-vitro anti-inflammatory effect of crude ethanol extract of *F. hispida* L. fruits was determined against denaturation of egg albumin.

3 ml of 5% egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (PBS, P^H 6.4) were used to make reaction mixture and 3 ml varying concentration of extract so that final concentration become 150, 300, 600 µg/ml. Phosphate buffer saline was used as negative control. The pH of the reaction mixtures was adjusted by 0.1N HCl. Then the mixtures were incubated at 37.2^{0}C for 15 minutes and heated at 70 ^{0}C for 5 minutes. After cooling and filtration, the absorbance was measured spectrophotometrically at 660 nm [23]. Diclofenac sodium (100µg/ml) was used as standard drug. The percentage inhibition of protein

denaturation was calculated by the following equation,

% Inhibition of protein denaturation= 100 × [1-(AB_2-AB_1)/ (AB_3-AB_1)

Where,

AB_1 = unheated test sample, AB_2 = heated test sample, AB_3 = heated control sample

2.6 Membrane stabilizing assay of extract

2.6.1 Preparation of blood samples

The blood samples were collected from the donors not consuming any NSAID drugs for two weeks. Na-citrate was used to prevent clotting. The blood samples were stored at 4^0C for 24 hours until use. It was subjected to centrifugation at 2500 rpm for 5 minutes. Then the packed cells were resuspended with equal volume of normal saline (pH 7.4) and centrifuged again. The process was repeated three times until the supernatants were clear. Finally, 10% red blood cell suspension was prepared with normal physiological saline and used immediately [24].

2.6.2 Hypotonic-solution induced inhibition of haemolysis of extract

By using 154 mM NaCl in 10 mM sodium phosphate buffer having P^H 7.4 was used to make isotonic buffer solution. The experiment was carried out in duplicate pairs. Stock RBC suspension 50 µl was mixed with 5 ml of the hypotonic solution containing the *F. hispida* L. fruits extract at concentration of 150, 300, 600 µg/ml, while the control sample was mixed with drug free solution. At room temperature the mixtures were incubated for 10 minutes and centrifuged for 5 minutes at 5000 rpm and the absorbance of the supernatant was measured at 540 nm. The total inhibition of haemolysis was then calculated (Table 3) by determining the % inhibition of haemolysis [25]. Diclofenac sodium 100µg/ml was used as a standard. The percent inhibition of haemolysis was calculated by the following equation:

% Inhibition of haemolysis = 100 × [1-(AB_2-AB_1)/ (AB_3-AB_1)]

Where, AB_1 = test sample in isotonic solution, AB_2 = test sample in hypotonic solution

AB_3 = control sample in hypotonic solution

2.7 Brine Shrimp Lethality Bioassay of Extract

Brine shrimp lethality bioassay [26] technique was used for determination of toxic properties of the plant extracts. DMSO sample solutions were applied against *Artemia Salina*. For the experiment, 5 gm of ethanol crude extract was dissolved in DMSO and solutions of varying concentrations (1280, 640, 320, 160, 80, 40, 20, 10, 5 µg/ml) were obtained by serial dilution technique. As a negative control Dimethyl sulphoxide (DMSO) solution was used.

3. Result

3.1 Phytochemical Screening

Qualitative chemical tests were performed for the ethanol fruits extract of *Ficus hispida* L. The results of various chemical tests are summarized in the (table 1).

Table 1. Phytochemical Screening of Ethanol Extractives of *Ficus Hispida* L. fruits.

Phytochemical constituents	Name of the test	Ethanol extract
Carbohydrates	Fehling's solution test Benedict's test	+ +
Tannins	Ferric Chloride test	+
Alkaloids	Mayer's test	-
Terpenes	Copper acetate test	+
Flavonoids	Lead acetate test	+
Glycosides	Legal's test	+
Saponins	Froth test Foam test	+ +
Steroids	Salkowski test , Liber-mann-Burchared test	+ +

(+) = Presence of constituents; (-) = Absence of constituents

3.2 Protein denaturation Activity of Ethanol Extract

In protein denaturation test, the crude ethanol extracts of *Ficus hispida* L. (600µg/ml) showed inhibition of protein denaturation 9.56% whereas for diclofenac sodium it was found to be 29.80% (Table 2). The ability of ethanol extract of *F. hispida* L. to inhibit hypotonic solution-induced protein denaturation was found to be mild.

Table 2. Evaluation of protein denaturation activity of ethanol extracts of fruits of *F. hispida* L.

Test sample	Concentration (µg/ml)	Absorbance (660 nm)	(%) Inhibition of protein denaturation
Negative Control (PBS)	0	2.101	0.00
Crude Extract	150	2.080	2.00
Crude Extract	300	2.000	5.03
Crude Extract	600	1.900	9.56
Standard (Diclofenac Sodium)	100	1.618	29.80

PBS= Phosphate buffer saline

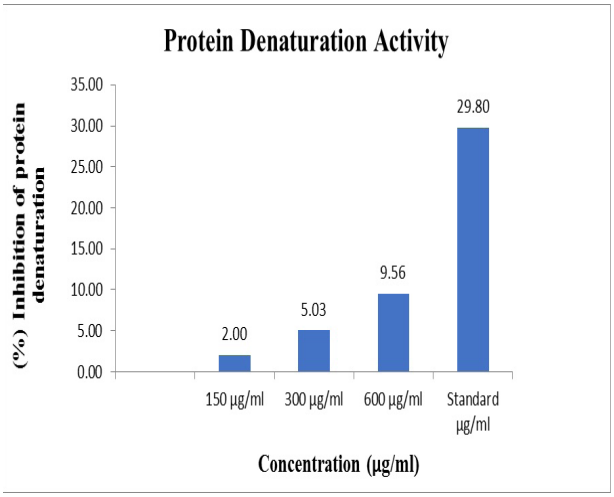

Fig. 1: Different percentages of protein denaturation at different concentration by ethanol extract of *F. hispida* fruits.

3.3 Membrane Stabilizing Activity

The in-vitro membrane stabilizing activities of crude ethanol extract of *F. hispida* L. fruits are given in the table 3.

Table 3. Percentage (%) inhibition of hypotonic solution-induced haemolysis of human erythrocyte membrane by standard and ethanol extract of *F. hispida* L.

Test sample	Concentration (µg/ml)	Absorbance (540 nm)	(%) Inhibition of haemolysis
			Hypotonic solution Induced
Negative Control (PBS)	0	0.135	0.00
Crude Extract	150	0.134	8.28
Crude Extract	300	0.127	17.16
Crude Extract	600	0.106	29.59
Standard (Diclofenac sodium)	100	0.027	91.72

PBS= Phosphate buffer saline

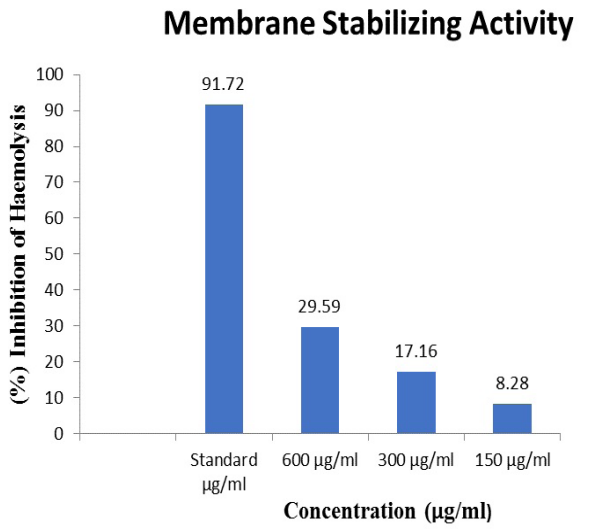

Fig. 2: Different percentages (%) inhibition of haemolysis at different concentration by ethanol extracts of *F. hispida* L. fruits.

At the concentration of 150, 300, 600µg/ml, the extractives of *Ficus hispida* L. fruits protected the haemolysis of RBC induced by hypotonic solution as compared to the standard diclofenac sodium (100 µg/ml). The crude ethanol extract (600µg/ml) inhibited 29.59% of haemolysis of RBCs induced by hypotonic solution as compared to 91.72% by diclofinac sodium (Table 3).

Table 4. The result of brine shrimp lethality bioassay of crude ethanol fruits extracts of *Ficus hispida* L.

Group	Conc. of sample (mg/ml)	LogC	No. of nauplii added	No. of death in each vial			Average Number of death	Mortality (%)	LC_{50}(µg/ml)
				1	2	3			
Control	20µl DMSO	0	10	0	0	0	0.00	0.00	0.00
Crude extract	5	0.69897	10	1	1	1	1.00	10.0	165.96
	10	1	10	1	2	1	1.33	13.3	
	20	1.30103	10	1	2	2	1.67	16.7	
	40	1.60206	10	2	1	3	2.00	20.0	
	80	1.90309	10	2	3	2	2.33	23.3	
	160	2.20412	10	4	5	3	4.00	40.0	
	320	2.50515	10	6	5	6	5.67	56.7	
	640	2.80618	10	8	8	7	7.67	76.7	
	1280	3.10721	10	9	10	9	9.33	93.3	

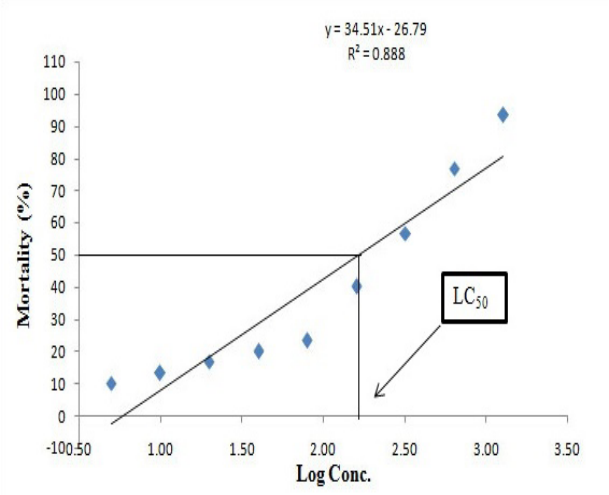

Fig. 3: Brine shrimp lethality test at different concentration of crude extract.

3.4 Brine Shrimp Lethality Assay of Crude Extracts

In brine shrimp lethality assay, the cytotoxic activities were screened using crude extracts. The concentration at which 50% mortality of brine shrimp nauplii occurred were determined and the LC_{50} was found to be 165.96 µg/ml for ethanol extract of *F. hispida* L.

4. Discussion

The present study assessed the protein denaturation, membrane stabilizing and cytotoxic activities of crude ethanol extract of *Ficus hispida* L. fruits. Carbohydrates, glycosides, tannins, terpenoids, saponins, flavonoids and steroids were identified by different chemical tests (Table 1). In protein denaturation condition, the extractives at 150, 300, 600 µg/ml inhibited moderate percentage of haemolysis of RBC such as 2.00%, 5.03% and 9.56 respectively while diclofenac sodium at 100 µg/ml showed 29.804% inhibition. At concentration 150, 300, 600µg/ml, the fruits extracts of *Ficus hispida* L. moderately protect the lysis of human erythrocyte membrane induced by hypotonic solution as compared to the standard diclofenac sodium at the concentration of 100µg/ml (Table-3). The crude ethanol extract (600µg/ml) inhibited 29.59% of haemolysis of RBCs induced by hypotonic solution as compared to 91.72% by diclofenac sodium; which confirmed that the plant had moderate membrane stabilizing activity. The extracts of the *Ficus hispida* L. fruits displayed a mild cytotoxic activity in dose dependent manner (Table 4). But ethanol extract of *F. hispida* L. fruits exhibited insignificant cytotoxicity.

5. Conclusion

The results obtained from the present study, it can be said that the ethanol fruits extract of *F. hispida* L. possesses moderate protein denaturation, membrane stabilizing and cytotoxic activities. Present work was a preliminary effort which will require further detailed investigation. However, further works are required in the laboratory to confirm these observed pharmacological actions.

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