Journal of Complementary and Alternative Medical Research

5(2): 1-13, 2018; Article no.JOCAMR.40429
ISSN: 2456-6276

Evaluation of Antioxidant, Cytotoxic, Antibacterial and Thrombolytic Activity of the Methanolic Extracts of *Ficus racemosa* Fruits

Mst. Shirajum Munira[1*], Md. Bahar Uddin[1], Md. Shariful Islam[1], Koly Aktar[1] and Abdul Muhit[2]

[1]Department of Pharmacy, Southeast University, Banani, Dhaka-1213, Bangladesh.
[2]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka, Bangladesh.

Authors' contributions

This work was carried out in collaboration between all authors. Author MSM designed the study, performed the statistical analysis wrote the protocol. Author MBU performed the experiments and Author MSI wrote the first draft of the manuscript. Author KA managed the literature searches and author AM carried out the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2018/40429
Editor(s):
(1) Arun Singh, Professor, Community Medicine, Institute: Rohilkhand Medical College & Hospital, Bareilly International University, India.
Reviewers:
(1) R.Jasmine, Bishop Heber College, India.
(2) S. Murugesan, Pachaiyappa's College, India.
(3) Nadia A. Rojano Vilchis, National Autonomous University of Mexico, México.
Complete Peer review History: http://www.sciencedomain.org/review-history/24099

Original Research Article

Received 28th January 2018
Accepted 6th April 2018
Published 11th April 2018

ABSTRACT

Aims: The aim of this study was to find out the antioxidant, cytotoxicity, antibacterial and thrombolytic activity of the methanolic extract of fruit of *F. racemosa.*
Place and Duration of Study: The study was carried out in August 2017 in the Department of Pharmacy, Southeast University, Dhaka, Bangladesh.
Materials and Methods: Total antioxidant capacity, DPPH free radical scavenging, total phenolic content, total flavonoid content activity was determined by several standard methods. Cytotoxicity activity was determined against brine shrimp nauplii by using the brine shrimp lethality bioassay. Vincristine sulphate is used as positive control. The antibacterial activity was evaluated using the disk diffusion technique here Kanamycin was used as standard. The thrombolytic activity was

**Corresponding author: E-mail: monira683382@gmail.com;*

determined by clot lysis method.
Results: The total antioxidant capacity of crude methanolic extract was also found very good compared to standard catechin. IC_{50} of BHT (standard) and crude methanol extract were 9.24 µg/ml and 11.36 µg/ml respectively. The presence of endogenous substances in *F. racemosa* fruits that may act as an antioxidant was established by measuring the total content of phenolic, flavonoid compounds. For the cytotoxicity test, in brine shrimp lethality bioassay, the LC_{50} value obtained was 12.34 µg/ml of the methanolic extract of the *F. racemosa* fruits. The zone of inhibition of fruit extracts of *F. racemosa* was in the range from 11 to 18 mm and the highest activity was observed against *Staphylococcus aureus* at 500 µg/disc having a zone of inhibition of 18 mm in diameter. For thrombolytic test, *F. racemosa* extracts showed poor clot lysis activity (21.1%) compared to the standard streptokinase (SK) whose clot lysis activity was 61.31%.
Conclusion: From the research it can be concluded that the methanolic extracts of *F. racemosa* fruits possess significant antioxidant, cytotoxic, antimicrobial, thrombolytic activity that could be a better treatment of diseases. So, further studies are recommended to isolate the exact compounds responsible this activity and their efficacy need to be tested.

Keywords: Antioxidant; cytotoxic; antimicrobial; thrombolytic; Ficus racemosa.

ABBREVIATIONS

DMSO : Dimethyl sulphoxide
LC50 : Lethal concentration
IC50 : concentration of an inhibitor
MEFR : Methanolic extract of fruits of F. racemosa
DPPH : 1, 1-diphenyl-2-picrylhydrazyl

1. INTRODUCTION

Medicinal plants have been associated with the human health care system from time immemorial, particularly among tribal [1]. Medicinal plants has assumed massive importance in present days, due to the tremendous developments in the field of allopathic medicines during the 20th century [2]. At present people are being bombarded with thousands of unhealthy products, the level of sensibility in front of diseases is very high [3]. The use of medicinal plants can represent the best solution [3]. *Ficus racemosa* (Linn.) (Family Moraceae) commonly Bengali name as Jagadumur is a very common plant which is used in our country for several purposes. *F. racemosa* is an important medicinal plant, found in India, Australia, and Southeast Asia. *F. racemosa* reduces blood glucose concentration due to the presence of β-sitosterol and many active constituents that have been isolated from various parts of this plant possess useful pharmacological activities [4]. *F. racemosa* plant is a medium-sized to large deciduous, sometimes evergreen tree with spreading crown and white latex, leaves 7.5-10 cm long, ovate-oblong, entire, tapering to a bluntish point at the apex, bark is reddish gray, grayish green, soft surface, uneven and often cracked, 0.5-1.8 cm thick, fruits receptacles are 2-5 cm in diameter, pyriform, in large clusters, large branches, roots are long and brownish in color [5]. *F. racemosa* traditionally used as an antidiuretic effect [6]. The roots are widely used for the treatment of hydrophobia, galactagogue, gynecological disorders [7] the fruits are effective against menorrhagia, leprosy, leucorrhoea, blood disorders, intestinal worms, burns, dry cough etc. [8] the leaf buds are used in skin infection, leaves are active against wound washing and healing [8]. *F. racemosa* has multiple pharmacological activities that include antidiabetic [9-12], antioxidant [13] anti-inflammatory [14,15], antidiarrheal [16] antibacterial [17], hypolipidemic [18] hepatoprotective [19,20] antitussive [21], antiulcer [22] anthelmintic [23] antidiuretic [24], anticancer [25] and memory enhancing activity [26]. As far our knowledge, no reports on fruits have been found by the literature survey on antioxidant, cytotoxic, antimicrobial and thrombolytic activities. So aim of the present work was to investigate the antioxidant, cytotoxic, antimicrobial and thrombolytic activities on *F. racemosa* fruits.

2. METHODS AND MATERIALS

2.1 Collection of Sample and Preparation of Extract

Ficus racemosa fresh fruits were collected from Comilla, Bangladesh, 2016 and identified by an expert taxonomist. A voucher specimen was submitted to the national herbarium, Mirpur, Dhaka, Bangladesh and accession number is 45881. About 650 mg of dried and

powdered plant materials obtained from the plant fruits were soaked in 3.5 liter of methanol in an amber glass container for about 14 days at room temperature with occasional shaking. After 14 days, the solution was filtered using cotton filter and Whitman's filter paper number 1. The filtrates were concentrated to afford solid masses by using a rotary evaporator [27,28]. Finally used for the experimental purpose.

2.1.1 Drugs and chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), catechin (CA), butylated hydroxytoluene (BHT), ascorbic acid (AA), $AlCl_3$, sodium phosphate, methanol, ammonium molybdate, was purchased from Sigma Chemical Co. (St. Louis, MO, USA); potassium acetate, HCl, H_2SO_4 were purchased from Sigma-Aldrich, Folin-ciocalteus's phenol reagent, sodium carbonate were obtained from Merck (Dam-stadt, Germany), Streptokinase (standard) 1500000 IU (Durakinase) was purchased from the local market. Other chemicals were obtained from local sources and were of analytical grade.

2.1.2 Phytochemical screening

Phytochemical screening of the fruit extract was tested for the presence of active principles such as alkaloids, flavonoids, tannins, proteins, etc. using the standard procedures by Rohit Kumar Bargah [29,30].

2.1.2.1 Test for tannins (Braymer's Test)

2 ml of the extract was stirred with 2 ml of distilled water and 2-3 drops of $FeCl_3$ (5%) solution were added. The formation of green color precipitate indicates the presence of tannins. Observation: Green color precipitate indicates the presence of tannins.

2.1.2.2 Test for flavonoids

1 ml of the extract, 1ml of 10% lead acetate $(Pb(OAc)_4)$ solution is added [9]. Observation: Yellow color precipitate was taken as a positive result for flavonoids.

2.1.2.3 Test for terpenoids

2 ml of the extract was dissolved in 2 ml of chloroform $(CH_3CO)_2O$ and evaporated to dryness. 2 ml of concentrated sulphuric acid (H_2SO_4) was added and heated for about 2 min. Observation: Deep red color indicates the presence of terpenoids.

2.1.2.4 Test for steroids (Salkowski Test)

2 ml of the extract was mixed with a few drops of acetic anhydride, boiled and colored. 2 ml concentrated sulphuric acid was then added from the sides of the test tube. Observation: The formation of reddish brown ring at the junction of two layers and green color of the upper layer and the formation of deep red color in the lower layer would indicate a positive test for steroids.

2.1.2.5 Test for phlobatannins (Precipitate Test)

2 ml of the extract was added to 2 ml of HCl (1%) and the extract was boiled. Observation: Deposition of a Red precipitate was taken as an indication of presence of phlobatannins.

2.1.2.6 Test for carbohydrates (Molisch's Test)

2 ml of the extract was treated with 2 drops of ethanolic αnaphthol (20%) solution in a test tube. Observation: Formation of the reddish violet ring at the junction indicates the presence of carbohydrate.

2.1.2.7 Test for coumarins

2 ml of the extract was added to 3 ml of NaOH (10%). Observation: Formation of the yellow color indicates the presence of coumarins.

2.1.2.8 Test for alkaloids (Hager's Test)

3 ml of the extract solution was treated with a few drops of Hager's reagent (saturated picric acid solution). Observation: Presence of alkaloids confirmed by the formation of a yellow coloured precipitate.

2.1.2.9 Test for proteins (Xanthoproteic Test)

The extracts were treated with a few drops of conc. nitric acid. Observation: Formation of yellow color indicates the presence of proteins.

2.1.2.10 Test for anthraquinones (Borntrager's Test)

3 ml of the extract was treated with 3 ml of benzene and then 5 ml aqueous NH_3 (10%) was added in a test tube. Observation: After shaking, change in color of the aqueous layer was observed. Pink, violet or red color in the aqueous layer indicated the presence of anthraquinones.

2.1.2.11 Test for Anthocyanins

2 ml of the extract was treated with 2 ml of HCl (2N) and then added NH_3 in a test tube. Observation: formation of pinkish red to bluish violet color indicates the presence of anthocyanins.

2.1.2.12 Test for glycosides (Keller Killiani Test)

Test solution was treated with a few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added. Observation: Formation of two layers. Lower reddish brown layer and upper acetic acid layer which turn a bluish green would indicate a positive test for glycosides.

2.1.2.13 Test for saponins (Foam Test)

5 ml of the extract was shaken vigorously with an equal volume of distilled water in a test tube and the mixture was warmed. Observation: The formation of emulsion forms or stable foam was taken as an indication of the presence of saponins.

2.1.2.14 Test for phenols

To 1 ml of the extracts of sample, 2 ml of distilled water, followed by a few drops of 10% aqueous ferric chloride solution was added. Observation: Formation of blue or green color indicated the presence of phenols.

2.2 Antioxidant Evaluation

2.2.1 Determination of total antioxidant capacity

Total antioxidant capacity of samples was determined by the method Prieto et al. [31] with some modifications. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH.0.5 mL of plant extract /standard at different concentrations (7.825–250 µg/mL) was mixed with 3 mL of reaction mixture containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate was added into the test tubes. The test tubes were incubated at 95ºC for 10 min to complete the reaction. Then, the absorbance of the solution was measured at 695 nm using a spectrophotometer against a blank solution to room temperature. Catechin was used as standard, a reference sample. A typical blank solution contained 3 mL of the reaction mixture and the appropriate volume (500 µl) of the same solvent used for the samples/standard, and it was incubated under the same conditions as the rest of the sample solution. The increased absorbance of the reaction mixture introduces increased total antioxidant capacity. We used standard/samples at six different concentration ranges from (7.825 to 250 µg/mL) for each antioxidant assay. The experiment was repeated three times at each concentration.

2.2.2 DPPH radical scavenging assay

DPPH was used to evaluate the free radical scavenging assay as described by Desmarchelier et al. [32]. The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. Resulting, DPPH produces purple/violet color in methanol solution and fades to shades of yellow color in the presence of antioxidants. 2 mL of methanol solution of plant extract or standard sample at different concentration (3.906–150 µg/mL) was mixed with 3 mL of methanol solution of DPPH was added into the test tube. The test tube was incubated at room temperature for 30 minutes in dark place to complete the reaction. Then the absorbance of the solution was measured at 517 nm using a spectrophotometer against a blank solution. A typical blank solution contained all reagents except plant extract or standard solution. The percentage (%) inhibition activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = \{(A_0 - A_1)/A_0\} \times 100$$

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. Then % of inhibition was plotted against concentration, and from the graph IC_{50} was calculated. The experiment was repeated three times at each concentration. The experiment was repeated three times at each concentration.

2.2.3 Determination of total phenolic

Total phenolic content of extract of *F. racemosa* was determined by the modified Folin-Ciocalteu reagent method described by Singleton et al. [33]

ascorbic acid as standard. 0.5 mg of crude extract or standard of different concentration solution was taken in a test tube, 2.5 ml of Folin – ciocalteu (Diluted 10 times with water) reagent solution was added into the test tube, 2.5 ml of Sodium carbonate (7.5%) solution was added into the test tube, the test tube was incubated for 1 hour at 25ºC temperature, then the absorbance of the solution was measured at 760 nm using a spectrophotometer against blank. Using the absorbance of the sample, total phenolic content is measured by using the following equation:

$$C = (c \times V)/m.$$

Where, C is the total content of phenolic compounds, mg/g plant extract, in AAE. c is the concentration of ascorbic acid established from the calibration curve, mg/ml. V is the volume of extract, ml. m = the weight of different pure plant extracts, gm. The experiment was repeated three times at each concentration.

2.2.4 Determination of total flavonoids

Total flavonoid content of extract of *F. racemosa* was determined by using aluminum chloride colorimetric assay described by Zhisen et al. [34] with some modifications. 1.0 ml of plant extract or standard of different concentration solution was taken in a test tube, 3 ml of methanol was added into the test tube, 200 µl of 10% aluminum chloride solution was added into the test tube, 200 µl of 1M potassium acetate solution was added into the test tube, 5.6 ml of distilled water was added into the test tube, The test tube was then incubated at room temperature for 30 minutes to complete the reaction, Then the absorbance of the solution was measured at 420 nm using a spectrophotometer against blank, total content of flavonoid compounds in plant extracts in ascorbic acid equivalents was calculated by using equation:

$$C = (c \times V)/m$$

Where, C is the total content of flavonoid compounds, mg/g plant extract, in AAE. c is the concentration of ascorbic acid established from the calibration curve, mg/ml. V is the volume of extract, ml. m is the weight of pure plant extracts, gm. The experiment was repeated three times at each concentration.

2.3 Cytotoxicity Evaluation

The brine shrimp lethality bioassay was determined by the method reported by Meyer and Zhao et al. [35,36]. 4 mg of each of the extract were dissolved in DMSO and solutions of various concentrations such as 400 µg/ml to 0.781 µg/ml were obtained by the serial dilution technique. Standard Vincristine Sulfate was used as the positive control and DMSO was used as the control, respectively.Next ten matured shrimps were taken to each of the experimental vials and the control vial. The number of the nauplii that died after 24 hrs was counted and the LC_{50} was calculated from the regression equation, obtained from the logarithm of sample concentration versus percentage mortality of the shrimp nauplii. The experiment was repeated three times at each concentration.

2.4 Antimicrobial Test

The disk diffusion method was determined by the method of Barry A.L. [37] against six microorganisms. The microbial strains used for the experiment were collected from the University of Jahangirnagar, Savar, Dhaka, Bangladesh. The extracts were dissolved in methanol as required. Applied to sterile filter paper in 250 and 500 µg/disc and cautiously dried to evaporate the remaining solvent. Standard antibiotics, Kanamycin (30 mg/disc) was used as positive control. An eextract of the collected plants was tested against *Micrococcus luteus, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae.* The antibacterial activities of the extracts were ascertained by measuring the respective zone of inhibition in millimeters. The experiment was repeated three times at each concentration.

2.5 Thrombolytic Test

In vitro clot lysis activity of the leaves of *F. racemosa* was carried out according to the method of Prasad et al. [38] with minor modification. Aliquots (5 ml) of venous blood were drawn from healthy volunteers who were distributed in ten different pre weighed sterile vials (1 ml/vial) and incubated at 37°C for 45 minutes. After clot formation, the serum was completely removed without disturbing the clot and each vial having clot was again weighed to determine the clot weight (clot weight = weight of clot containing vial – weight of vial alone). To each vial containing pre-weighed clot, 100 µl aqueous solutions of different partitionates along with the crude extract was added separately. As a positive control, 100 µl of streptokinase (SK) and as a negative non thrombolytic control, 100

µl of distilled water were separately added to the control vials. All the vials were then incubated at 37ºC for 90 minutes and observed for clot lysis. Therefore, after incubation, the released of fluid was removed and vials were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

% clot lysis = (Weight of the lysis clot / Weight of clot before lysis) × 100

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Here (+) means the component is present and (-) means the component is absent. The presence of Tannins, Flavonoids, Terpenoids, Carbohydrates, Coumarins, Alkaloids, Proteins, Phenols, Glycosides, Saponins and the absences of Quinones, Anthocyanins, Anthraquinones, and Steroids has been shown qualitatively in the Table 1.

3.2 Total Antioxidant Capacity

The antioxidant potential of the methanolic extracts of *F. racemosa* was estimated from their ability to reduce the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex with a maximal 695 nm. The total antioxidant activity of plant extracts and (+) catechin (standard) was depicted in (Table 2) and in (Fig. 1). As shown in the Fig. 1 crude methanolic extract showed considerable antioxidant activity compared to (+)-catechin (Standard). The activity was less than that of catechin. At the concentration 62.50 µg/ml, the absorbance of crude methanol extract and (+)-catechin were 0.590 and 0.875 respectively. The extract was found to increase the total antioxidant activity with the increasing concentration of the extract. At 250 µg/ml, the absorbance of crude methanol extract and (+)-catechin were 1.677 and 2.457 respectively.

Table 1. Phytochemical test results of crude methanolic extract of *F. racemosa* (fruits)

Phytochemical tests	Crude methanol extract
Tannins	+
Flavonoids	++
Terpenoids	+
Steroids	-
Phlobatannins	-
Carbohydrates	++
Coumarins	+
Alkaloids	++
Proteins	++
Anthraquinones	-
Anthocyanins	-
Glycosides	+
Saponins	++
Phenols	++

Table 2. Total antioxidant activity of the crude methanol extract of *F. racemosa* and (+)-catechin (standard) at different concentrations

Name of sample	Conc. (µg/ml)	Absorbance			Absorbance mean ±STD
		A	b	c	
(+)-Catechin	7.825	0.052	0.053	0.055	0.053 ± 0.0012
	15.625	0.225	0.228	0.221	0.231 ± 0.0029
	31.25	0.723	0.720	0.718	0.425 ± 0.0020
	62.50	0.876	0.874	0.877	0.875 ± 0.0012
	125	1.461	1.463	1.469	1.464 ± 0.0033
	250	2.457	2.459	2.456	2.457 ± 0.0012
Crude methanol extract	7.825	0.011	0.013	0.010	0.0113 ± 0.0016
	15.625	0.206	0.203	0.208	0.205 ± 0.0020
	31.25	0.356	0.360	0.362	0.359 ± 0.0024
	62.5	0.592	0.588	0.591	0.590 ± 0.0017
	125	1.116	1.115	1.118	1.116 ± 0.0012
	250	1.679	1.671	1.682	1.677 ± 0.0046

3.3 DPPH Radical Scavenging Activity

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples including plant extracts. The DPPH antioxidants assay is based on the ability of 1, 1 diphenyl-2-picryl-hydrazyl (DPPH), and a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured by the change in absorbance and % of scavenging activity is calculated. The activity was increased by increasing the concentration of the sample extract. The antioxidant activity of the extracts of *F. racemosa* was evaluated by DPPH radical scavenging assays. The results of DPPH radical scavenging assays of plant extracts and BHT (butylated hydroxytoluene) (sample) are given in (Table 3) and in (Fig. 2). IC_{50} of standard and crude methanol extract were 9.24 µg/ml and 11.36 µg/ml respectively.

3.4 Total Phenolic Content

Phenolic content of the methanol extract of fruits of *F. racemosa* were determined using Folin-Ciocalteu reagent. Phenolic content of the samples were calculated on the basis of the standard curve for ascorbic acid as shown in (Fig. 3). The results were expressed as mg of ascorbic acid equivalent (AAE)/gm of dried extract. The phenolic content of methanolic extract was 24.639 mg of AAE /gm of dried extract are represented in Table 4.

3.5 Total Flavonoid Content

Total flavonoids content of methanol extract (CME) was determined using much known aluminum chloride colorimetric method. Flavonoid content of the sample was calculated on the basis of the standard curve for ascorbic acid as shown in (Fig. 4). The results were expressed as mg of ascorbic acid equivalent (AAE)/gm. of extract. The flavonoid content of methanol extract was 177.73 mg of AAE/gm of dried extract are shown in Table 5.

Table 3. DPPH radical scavenging activity of the crude methanol extract of *Ficus racemosa* and BHT (Standard) at different concentrations

Name of sample	Conc. (µg/ml)	% of scavenging			% of scavenging mean ± STD	IC_{50} (µg/m)
		a	b	C		
BHT	15.626	84.46	84.49	84.53	84.49 ±0.035	
	31.25	88.76	88.71	88.82	88.76 ±0.055	9.24
	62.5	92.45	92.54	92.41	92.46 ±0.066	
	125	93.65	93.76	93.62	93.67± 0.073	
	250	95.44	95.57	95.53	95.51 ±0.066	
	500	96.33	96.40	96.46	96.39 ±0.065	
Crude methanol extract	15.626	68.67	68.76	68.73	68.72±0.045	
	31.25	69.45	69.54	69.55	69.51 ±0.055	
	62.5	70.43	70.49	70.63	70.51 ±0.102	11.36
	125	73.45	73.55	73.63	73.54 ±0.090	
	250	76.23	76.31	76.27	76.27 ±0.040	
	500	79.47	79.55	79.61	79.54 ±0.070	

Table 4. Total phenolic content of crude methanolic extract of *Ficus racemosa* fruits

Sample	Concentration (mg/ml)	Absorbance	Total phenolic content (mg of AAE/gm of dried extract)
MFFR	2	1.292	24.631

Table 5. Total flavonoid content of crude methanolic extract of *F. racemosa* fruits

Sample	Concentration (mg/ml)	Absorbance	Total flavonoid content (mg of AAE/g of dried extract)
MFFR	1	0.014	32.941

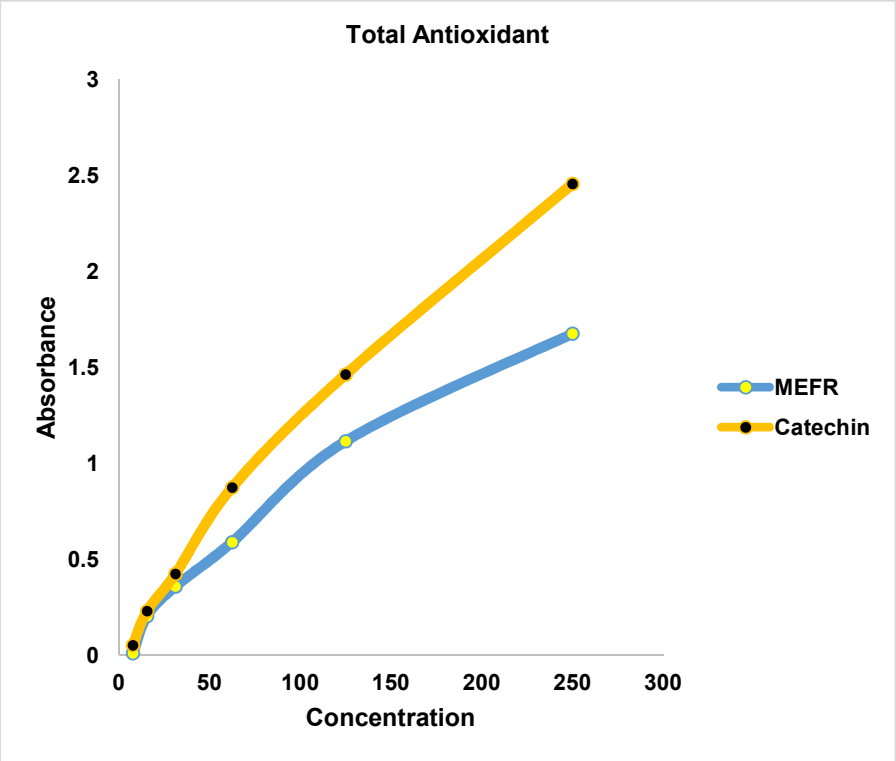

Fig. 1. Crude methanolic extract showed considerable antioxidant activity compared to (+)-catechin (Standard)

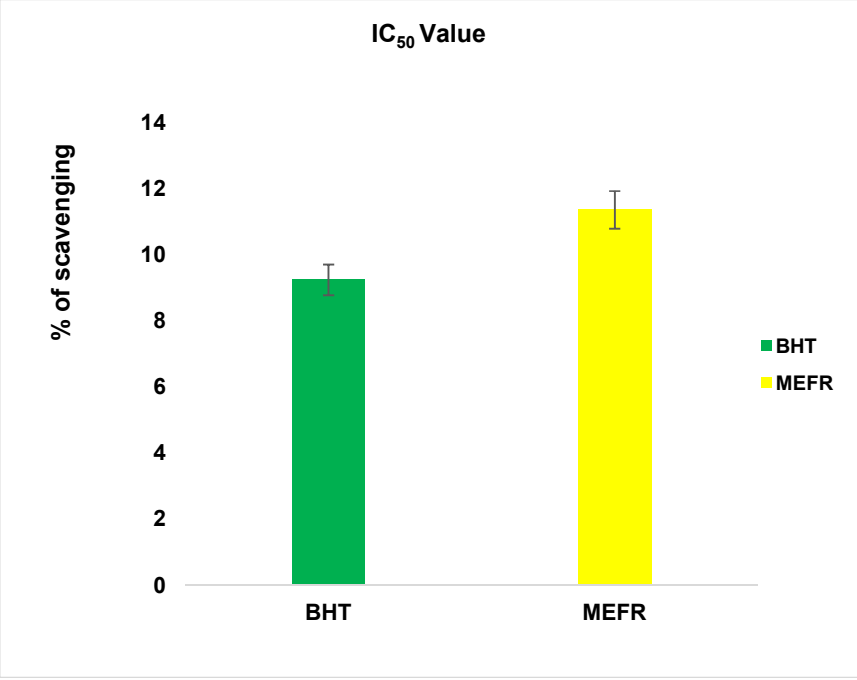

Fig. 2. Phenolic content of the samples were calculated on the basis of the standard curve for ascorbic acid

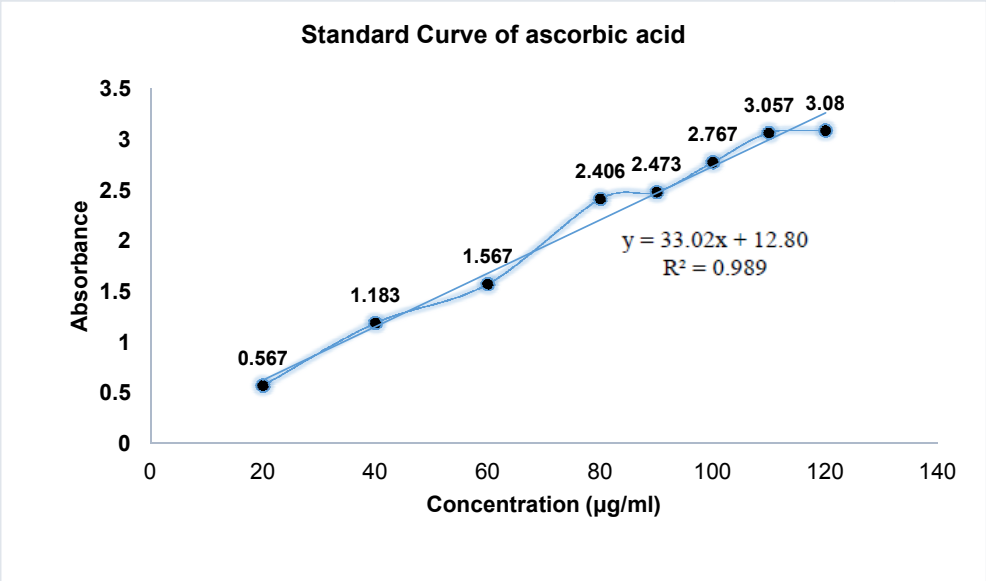

Fig. 3. Phenolic content of the samples were calculated on the basis of the standard curve for ascorbic acid

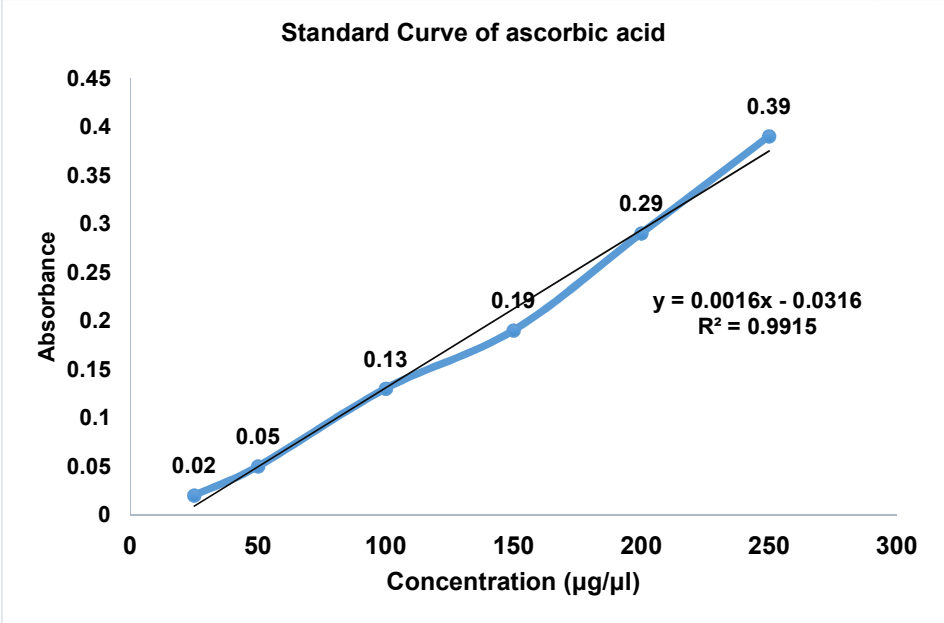

Fig. 4. Flavonoid content of the sample was calculated on the basis of the standard curve for ascorbic acid

3.6 Cytotoxicity Test

Methanolic extract of *F. racemosa* of the plant was tested and brine shrimp lethality bioassay using the following the procedure of Meyer [39]. This method was applied for the determination of toxic property of the extract. The LC_{50} values for standard vincristine sulphate, methanolic extract were found to be 13.38 µg/ml and 12.34 µg/ml, respectively, which indicate that the plant has potent cytotoxic effect.

3.7 Antimicrobial Test

At different concentrations (250 & 500 µg/disc) of methanolic extracts showed sustained activity

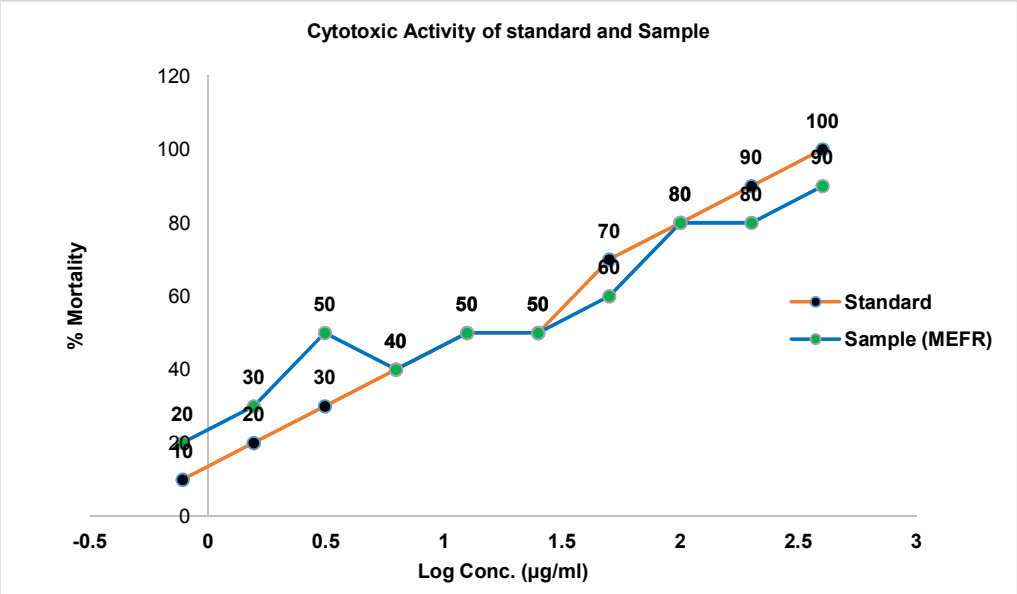

Fig. 5. Determination of LC_{50} values for standard Vincristine Sulphate and methanolic extract of leaves *F. racemosa* from linear correlation between logarithms of concentration versus percentage of mortality

Table 6. Zone of inhibition of standard and methanolic extract of *F. racemosa*

Gram positive bacteria			
Name of microorganism	**Conc. (30 µg/disc)**	**Zone of inhibition (mm)**	
		Methanolic extract	**Kanamycin (30 µg/disc)**
Micrococcus luteus	250	11	26
	500	14	
Bacillus cereus	250	7	29
	500	9	
Staphylococcus aureus	250	8	30
	500	18	
Gram negative bacteria			
Name of microorganism	**Conc. (30 µg/disc)**	**Methanolic extract**	**Kanamycin (30 µg/disc)**
Escherichia coli	250	8	30
	500	13	
Pseudomonas aeruginosa	250	6	27
	500	9	
Klebsiella pneumoniae	250	8	29
	500	14	

against all the tested bacterial strains. The highest activity observed was against *Staphylococcus aureus* at concentrations of 500 µg/disc having 18 mm in diameter. However, the fruit extracts of *F. racemosa* showed a broad-spectrum antibacterial activity with a zone of inhibition of 11 to 18 mm. The activity observed of standard drug, Kanamycin was found higher than these active concentrations showing 26-30 mm in diameter against all the tested bacterial strains. The results are given in the following table (Table 6).

3.8 Thrombolytic Activity

In present study, as a part of discovery cardio protective agents from natural sources, the extracts of *F. racemosa* fruits were assessed for thrombolytic activity and the results are presented in the following Fig. 6. Addition of

Table 7. Data analysis for thrombolytic activity of crude methanolic fruits extract of *F. racemosa*

Fractions	W_1mg	W_2 (mg)	W_3 (mg)	W_4 (mg)	W_5 (mg)	% of lysis (W_5/W_4)X 100%
MEFR1	813	1160	1087	347	73	21.1
MEFR2	805	1057	1025	252	32	12.7
Blank	815	1151	1130	336	21	6.26
SK	835	1520	1100	685	420	61.31

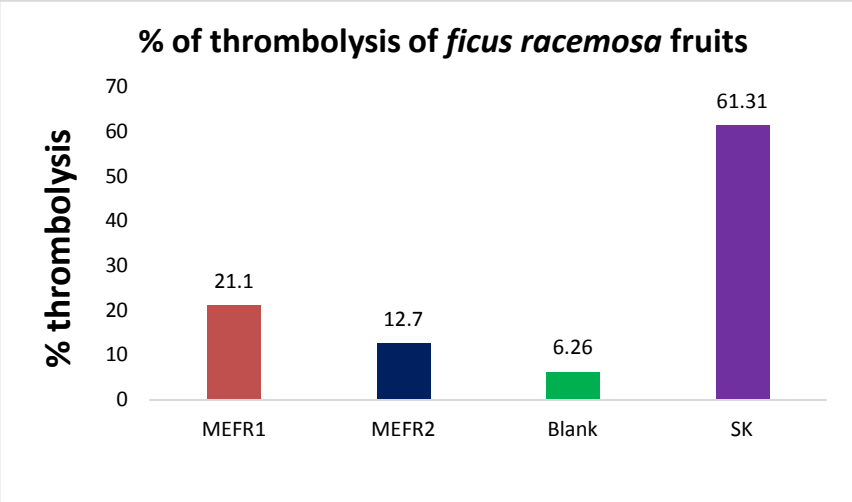

Fig. 6. Thrombolytic activity of crude methanolic fruits extract of *F. racemosa*

100 µl SK, a positive control (30,000 I.U.), to the clots and subsequent incubation for 90 minutes at 37ºC, showed 61.31% lysis of clot, the methanolic extract of fruits of *F. racemosa* (MEFR) exhibited thrombolytic activity 21.1% thrombolytic activity and distilled water was treated as negative control which exhibited a negligible percentage of lysis of clot (6.26%).

4. CONCLUSION

As the literature review suggests, the presence of several phytochemical compounds in *F. racemosa* makes the plant pharmacologically active. Consumption of antioxidant enriched fruits and vegetables is known to lower the risk of several diseases caused by free radical. The medicinal plant, *Ficus racemosa* L was analyzed for its chemical composition [40]. Chemical analysis showed that the plant is rich in nutrients, especially antioxidant compounds such as total phenol. Phytochemical screening showed that the methanolic extract contains the bioactive constituents such as tannins, saponins, phenols, flavonoids and terpenoids. The methanolic extract possesses cytotoxic activity that could be a better treatment in tumor as well as cancer. The study showed that, the extract showed low to moderate antimicrobial activity that could be a better treatment in antimicrobial infections. The extract showed low to moderate thrombolytic activity. However, studies are required in the higher animal model and subsequently on human subjects to prove efficacy as an antioxidant, cytotoxic, thrombolytic and antimicrobial agent. The medicinal values of the fruits of this plant may be due to the presence phytochemical constituent like tannins, saponins, phenols, flavonoids, alkaloid and terpenoids. So, further investigations are needed to isolate and identify the active compounds present in the plant extract and its various fractions and their efficacy need to be tested. It will help in the development of new, novel and safe drugs for the treatment of various diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The protocol of the experiment was approved by the animal ethics committee of the Department of

Pharmacy, Southeast University, Dhaka, Bangladesh. The animals care and health were maintained according to the guidelines of the National Institutes of Health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Morton J. Bignay. In: Fruits of warm climates. Julia F. Morton, Miami, FL. 1987; 210–212.
2. Oke JM, Hamburger MO. Screening of some Nigerian medicinal plants for antioxidant activity using 2, 2, diphenyl-picryl-hydrazyl radical. African Journal of Biomedical Research. 2002;5(1-2).
3. Al-Attar AM, Al-Taisan WA. Preventive effects of black seed (Nigella sativa) extract on sprague dawley rats exposed to diazinon. Australian Journal of Basic and Applied Sciences. 2010;4(5):957-68.
4. Yadav RK, Nandy BC, Maity S, Sarkar S, Saha S. Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*. Pharmacognosy Reviews. 2015;9(17):73.
5. Tasnim N. *In vitro* Pharmacological Investigations (cytotoxic, anti-microbial and anti-oxidant activity) fraction of dichloromethane of *Ficus Racemosa*. doctoral dissertation, East West University; 2014.
6. Shiksharthi AR, Mittal S. *Ficus racemosa*: Phytochemistry, traditional uses and pharmacological properties: A review. International Journal of Recent Advances in Pharmaceutical Research. 2011;4:6-15.
7. Joseph B, Raj SJ. Phytopharmacological and phytochemical properties of three ficus species-an overview. Int. J. Pharma. Bio. Sci. 2010;1:246-53.
8. Baslas RK, Agha R. Isolation of a hypoglycemic principle from the bark of Ficus glomerata Roxb. Himalayan Chem Pharm Bull. 1985;2(1):13-4.
9. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. Journal of Ethnopharmacology. 2003; 84(1):105-8.
10. Bhaskara Rao R, Murugesan T, Sinha S, Saha BP, Pal M, Mandal SC. Glucose lowering efficacy of *Ficus racemosa* bark extract in normal and alloxan diabetic rats. Phytotherapy Research. 2002;16(6):590-2.
11. Yadav RK, Nandy BC, Maity S, Sarkar S, Saha S. Phytochemistry, pharmacology, toxicology, and clinical trial of *Ficus racemosa*. Pharmacognosy Reviews. 2015;9(17):73.
12. Ahmed F, Urooj A. *In vitro* studies on the hypoglycemic potential of *Ficus racemosa* stem bark. Journal of the Science of Food and Agriculture. 2010;90(3):397-401.
13. Veerapur VP, Prabhakar KR, Parihar VK, Kandadi MR, Ramakrishana S, Mishra B, Rao S, Srinivasan KK, Priyadarsini KI, Unnikrishnan MK. *Ficus racemosa* stem bark extract: A potent antioxidant and a probable natural radioprotector. Evidence-Based Complementary and Alternative Medicine. 2009;6(3):317-24.
14. Mandal SC, Maity TK, Das J, Saba BP, Pal M. Anti-inflammatory evaluation of *Ficus racemosa* linn. Leaf extract. Journal of Ethnopharmacology. 2000;72(1-2):87-92.
15. Li RW, Leach DN, Myers SP, Lin GD, Leach GJ, Waterman PG. A new anti-inflammatory glucoside from *Ficus racemosa* L. Planta Medica. 2004;70(05): 421-6.
16. Mukherjee PK, Saha K, Murugesan T, Mandal SC, Pal M, Saha BP. Screening of anti-diarrhoeal profile of some plant extracts of a specific region of West Bengal, India. Journal of Ethnopharmacology. 1998;60(1):85-9.
17. Mandal SC, Saha BP, Pal M. Studies on antibacterial activity of *Ficus racemosa* linn. Leaf extract. Phytotherapy Research. 2000;14(4):278-80.
18. Sophia D, Manoharan S. Hypolipidemic activities of *Ficus racemosa* linn. Bark in alloxan induced diabetic rats. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(3):279-88.
19. Eshwarappa RS, Iyer S, Subaramaihha SR, Richard SA, Dhananjaya BL. Antioxidant activities of *Ficus glomerata* (moraceae) leaf gall extracts. Pharmacognosy Research. 2015;7(1):114.
20. Mandal SC, Maity TK, Das J, Pal M, Saha BP. Hepatoprotective activity of *Ficus racemosa* leaf extract on liver damage caused by carbon tetrachloride in rats. Phytotherapy Research. 1999;13(5):430-2.
21. Bhaskara Rao R, Murugesan T, Pal M, Saha BP, Mandal SC. Antitussive potential of methanol extract of stem bark of *Ficus*

racemosa linn. Phytotherapy Research. 2003;17(9):1117-8.
22. Rao CV, Verma AR, Vijayakumar M, Rastogi S. Gastroprotective effect of standardized extract of Ficus glomerata fruit on experimental gastric ulcers in rats. Journal of Ethnopharmacology. 2008; 115(2):323-6.
23. Chandrashekhar C, Latha K, Vagdevi H, Vaidya V. Anthelmintic activity of the crude extracts of *Ficus racemosa*. International Journal of Green Pharmacy. 2008;2(2): 100.
24. Ratnasooriya WD, Jayakody JR, Nadarajah T. Antidiuretic activity of aqueous bark extract of Sri Lankan *Ficus racemosa* in rats. Acta Biologica Hungarica. 2003;54(3-4):357-64.
25. Sukhramani PS, Vidyasagar G, Patel PM. *In-vitro* screening of *Ficus racemosa* for anticancer activity. Research Journal of Pharmacognosy and Phytochemistry. 2013;5(3):119-22.
26. Ahmed F, Chandra JN, Manjunath S. Acetylcholine and memory-enhancing activity of *Ficus racemosa* bark. Pharmacognosy Research. 2011;3(4):246.
27. Jeff ery GH, Basse J, Mendham J, Denney RC. Vogel's textbook of quantitative chemical analysis. 5th ed. England: Longman Group UK Ltd. 2000;161.
28. Haque M, Jahan T, Rashid MA. Antibacterial and cytotoxic activities of *Alocasia fornicata* (Roxb.) Int J Nutr Pharmacol Neural Dis. 2014;4:29-33.
29. Rohit kumar Bargah. Preliminary test of phytochemical screening of crude ethanolic and aqueous extract of *Moringa pterygosperma Gaertn*. Journal of Pharmacognosy and Phytochemistry. 2015;4(1):07-09.
30. Trease GE, Evans WC. Pharmacognosy 11th edn, Baillere Tindoll, London. 1989; 45-50.
31. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Analytical Biochemistry. 1999; 269(2):337-41.
32. Desmarchelier C, Novoa Bermudez MJ, Coussio J, Ciccia G, Boveris A. Antioxidant and prooxidant activities in aqueous extracts of argentine plants. International Journal of Pharmacognosy. 1997;35(2): 116-20.
33. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture. 1965; 16(3):144-58.
34. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999;64:555–9.
35. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica. 1982;45(05):31-4.
36. Zhao G, Hui Y, Rupprecht JK, McLaughlin JL, Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of Asimina triloba. Journal of Natural Products. 1992;55(3):347-56.
37. Barry AL. Principle and practice of microbiology. Lea and Fabager, Philadelphia; 1976.
38. Prasad S, Kashyap RS, Deopujari JY, Purohit HJ, Taori GM, Daginawala HF. Effect of fagonia arabica (Dhamasa) on *In vitro* thrombolysis. BMC Complementary and Alternative Medicine. 2007;7(1):36.
39. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DJ, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica. 1982;45(05):31-4.
40. Nipa Rani Das. Cytotoxic, antimicrobial & antioxidant activity of aqueous fraction of *Ficus racemosa*. East West University; 2014.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24099