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

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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.

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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
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 sulfoxide
- **LC$_{50}$**: Lethal concentration
- **IC$_{50}$**: 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 sensibility in front of diseases is very high [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* 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 anti-diuretic 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], antieulcer [22] anthelmintic [23] antiuideretic [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), AlCl₃, sodium phosphate, methanol, ammonium molybdate, was purchased from Sigma Chemical Co. (St. Louis, MO, USA); potassium acetate, HCl, H₂SO₄ were purchased from Sigma-Äldrich, Folin-ciocalteus’s phenol reagent, sodium carbonate were obtained from Merck (Damstadt, 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 FeCl₃ (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, 1 ml 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 (CH₂CO)₂O and evaporated to dryness. 2 ml of concentrated sulphuric acid (H₂SO₄) was added and heated for about 2 min. Observation: Deep red color indicates the presence of terpenoids.

2.1.2.4 Test for steroids (Saikowsk’s 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 NH₃ (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 NH₃ 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 0.8 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate was added into the test tubes. The test tubes were incubated at 95°C 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. 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:

\[
\text{% DPPH radical scavenging activity} = \left(\frac{A_0 - A_1}{A_0}\right) \times 100
\]

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 IC₅₀ 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 25°C 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:

\[ C = \frac{(c \times V)}{m} \]

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:

\[ C = \frac{(c \times V)}{m} \]

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<sub>50</sub> 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 Jahangimagar, 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 extract 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-weighted 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 37°C 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:

$$\% \text{ clot lysis} = \left( \frac{\text{Weight of the lysis clot}}{\text{Weight of clot before lysis}} \right) \times 100$$

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 absence 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 methanol extract of *F. racemosa* (fruits)

<table>
<thead>
<tr>
<th>Phytochemical tests</th>
<th>Crude methanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>++</td>
</tr>
<tr>
<td>Coumarins</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>++</td>
</tr>
<tr>
<td>Proteins</td>
<td>++</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Phenols</td>
<td>++</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Name of sample</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th>Absorbance mean ±STD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>b</td>
</tr>
<tr>
<td>(+)-Catechin</td>
<td>7.825</td>
<td>0.052</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>15.625</td>
<td>0.225</td>
<td>0.228</td>
</tr>
<tr>
<td></td>
<td>31.25</td>
<td>0.723</td>
<td>0.720</td>
</tr>
<tr>
<td></td>
<td>62.50</td>
<td>0.876</td>
<td>0.874</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1.461</td>
<td>1.463</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2.457</td>
<td>2.459</td>
</tr>
<tr>
<td>Crude methanol extract</td>
<td>7.825</td>
<td>0.011</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>15.625</td>
<td>0.206</td>
<td>0.203</td>
</tr>
<tr>
<td></td>
<td>31.25</td>
<td>0.356</td>
<td>0.360</td>
</tr>
<tr>
<td></td>
<td>62.5</td>
<td>0.592</td>
<td>0.588</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>1.116</td>
<td>1.115</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>1.679</td>
<td>1.671</td>
</tr>
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</table>
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\textsubscript{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 methanol 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

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

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/ml)</th>
<th>Absorbance</th>
<th>Total phenolic content (mg of AAE/gm of dried extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFFR</td>
<td>2</td>
<td>1.292</td>
<td>24.631</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (mg/ml)</th>
<th>Absorbance</th>
<th>Total flavonoid content (mg of AAE/g of dried extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MFFR</td>
<td>1</td>
<td>0.014</td>
<td>32.941</td>
</tr>
</tbody>
</table>
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
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
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*

<table>
<thead>
<tr>
<th>Fractions</th>
<th>$W_1$ (mg)</th>
<th>$W_2$ (mg)</th>
<th>$W_3$ (mg)</th>
<th>$W_4$ (mg)</th>
<th>$W_5$ (mg)</th>
<th>% of lysis ($W_5/W_4$) x 100%</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEFR1</td>
<td>813</td>
<td>1160</td>
<td>1087</td>
<td>347</td>
<td>73</td>
<td>21.1</td>
</tr>
<tr>
<td>MEFR2</td>
<td>805</td>
<td>1057</td>
<td>1025</td>
<td>252</td>
<td>32</td>
<td>12.7</td>
</tr>
<tr>
<td>Blank</td>
<td>815</td>
<td>1151</td>
<td>1130</td>
<td>336</td>
<td>21</td>
<td>6.26</td>
</tr>
<tr>
<td>SK</td>
<td>835</td>
<td>1520</td>
<td>1100</td>
<td>685</td>
<td>420</td>
<td>61.31</td>
</tr>
</tbody>
</table>

**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 37°C, 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, alkaloid 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


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