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Full Length Research Paper

In vitro thrombolytic activity, antioxidant and cytotoxic properties of fruit extracts of *Ficus erecta* (Thunb.)

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The aim of the study was to evaluate different biological properties of fruits extracts of *Ficus erecta* (Thunb.). The concentrated methanolic extracts and Kupchan partitions were screened for thrombolytic activity, whereas streptokinase was used as standard antioxidant property by evaluating free radical scavenging effect and determining total phenolic content and cytotoxic activity by brine shrimp lethality bioassay. The highest thrombolytic activity was exhibited by carbon tetrachloride soluble partition (CTP) of the plant (27.53±0.41%) against the standard streptokinase (65.16±0.064%). The IC_{50} value of the petroleum ether soluble partition (PEP) was found to be 7.35±0.08 µg/ml, whereas standard ascorbic acid demonstrated IC_{50} value of 5.80±0.22 µg/ml. The petroleum ether soluble partition (PEP) also exhibited highest phenolic content (43.69±1.4 mg of GAE/100 g of extractives). A correlation was calculated between total phenolic content and free radical scavenging activity of *F. erecta* which showed positive result having correlation coefficient (R^2) of 0.779. Among the LC_{50} values of the plant material, petroleum ether soluble partition (PEP) exhibited the lowest value of 1.20±0.028 µg/ml as compared to the standard vincristine sulphate (0.45±0.003 µg/ml). The present study showed that, the plant *F. erecta* demonstrated strong cytotoxic and antioxidant property and moderate thrombolytic activity.

Key words: *Ficus erecta*, thrombolytic, antioxidant, free radical scavenging, phenolic content, brine shrimp lethality bioassay, streptokinase, vincristine sulphate, acetyl salicylic acid.

INTRODUCTION

Plants and their extracts have been used for their healing properties for a long time now. It predates written human history. Phytochemicals exerts their effect on human body by a process similar to the chemical constituents of

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conventional drugs. This enables herbal medicines to have beneficial pharmacology, but also gives them the same potential as conventional pharmaceutical drugs to cause harmful side effects (Tapsell et al., 2006; Lai and Roy, 2004).

Fibrinolytic drugs are used to dissolve (lyse) blood clots which can occur in any vascular bed. A fibronolytic drug dissolves thrombin from the obstructed coronary arteries and inhibits necrosis by restoring normal blood circulation in myocardium as well as boost recovery (Laurence et al., 1992). Although they are efficient but some significant shortcomings were reported earlier including limited fibrin specificity, limited efficacy and large dose requirement and increasing hemorrhage in patients. To minimize the drawbacks of these drugs, many projects are being undertaken to develop recombinant variants with better efficacy (Marder, 1993).

Free radicals are highly reactive particles which are produced as by-products of metabolic processes or by radiation and can start chain reactions. These chain reactions can cause disintegration of cell membranes and cellular compounds (Leong and Shui, 2002). Theses damages inside the cells have been correlated to various disorders including arthritis, cancer, Alzheimer's disease, atherosclerosis and diabetes (Clancy and Birdsall, 2013, Karthikeyan et al., 2011). Also, the free radicals are held responsible for many age-related diseases (Harman D, 2009). Antioxidants are crucial chemical compounds that have the capability to give protection against such damages caused by oxidative stress induced by free radicals (Doss et al., 2012).

Brine shrimp lethality bioassay is a very useful method due to the low cost, ease of performing and the commercial availability of inexpensive brine shrimp eggs (Meyer et al., 1982). This assay is assumed as an indicator for general toxicity and after the isolation of active compounds more sophisticated bioassays can be performed for the determination of toxicity (McLaughlin et al., 1998).

Ficus erecta is a deciduous or semi-deciduous shrub or tree which can grow from 2-7 meters tall. The plant is sometimes harvested for food and fiber**.** Its flowers grow inside hypanthodium. It is a typical plant of fig species. Fig trees usually turn orange-red after maturity and look very brilliantly beautiful. Fruits contain milky white saps. Thus, it has a folk name "milky banyan". Leaves of the plant turn completely oval after maturity. Red new leaves and leaf veins are very attractive. Roots, stems, leaves and fruits all have medical effects. Stem barks can be used for paper or fiber extraction (Berg et al., 1989; Kislev et al., 2006).

In the current study, the methanolic extract of the fruit and its various organic soluble partitions were investigated for the thrombolytic, cytotoxic and antioxidant activity in terms of total phenolic content and free radical scavenging activity of *F. erecta* for the first time. To understand the relation between the free radical scavenging and total phenolics of the extractives, an attempt has been taken to establish a correlation.

MATERIALS AND METHODS

The fruits of *F. erecta* were collected from Meherpur, Khulna, Bangladesh in the month of June, 2015 and identified in the Bangladesh National Herbarium, Dhaka, where a voucher specimen (No. 41614) has been mentioned for future reference.

Preparation of extract

The fruits were sliced and air dried for 30 days. The sun dried fruits were powdered and the powdered material (400 g) was taken in a cleaned, amber color reagent bottle (5 liters) and macerated in 2.5 L of methanol for 15 days. The whole mixture was then filtered with a fresh cotton plug and finally through a Whatman No. 1 filters paper. The filtrate was then allowed to evaporate at ambient temperature until approximately 70% solvent was evaporated. The concentrated extract was then partitioned by modified Kupchan method (Van Wagenen et al., 1993). The crude methanolic (MEP) extracts and the resulting partitionates such as pet-ether (PEP), chloroform (CHP), carbon tetrachloride (CTP) and aqueous (AQP) soluble partition were subjected to various experimental studies.

Streptokinase (SK)

Lyophilized Altepase (Streptokinase) Eppendorf tube of 15, 00,000 I.U., which was a generous gift from Beacon Pharmaceutical Ltd., Bangladesh, was collected and 5 ml sterile distilled water was added and mixed properly. From this stock suspension, 100 µl (30,000 I.U) was used for *in vitro* thrombolytic study.

Blood sample

Ten healthy human volunteers who were not on oral contraceptives or anticoagulant therapy were chosen. 1 ml of the blood collected from the volunteers was transferred to each of the weighed sterile vials.

Thrombolytic activity

The thrombolytic activity was evaluated by the method developed by Prasad et al. (2006) with slight modification and streptokinase (SK) was used as the standard. The serum was removed after clot formation and the clot weight was determined. To each vial, 100 µl aqueous solutions from each of the partitionates along with the crude extracts were added. As a positive control, 100 µl of streptokinase (SK) was added to the vial and in the vial for negative control, 100 µl of distilled water was added. After incubating the control and sample vial for 90 min at 37°C fluid was removed and vials were again weighed to calculate the amount of lysis of clot and percentage of clot lysis was computed.

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

Total phenolic content analysis

The total phenolic content of *F. erecta* extractives were evaluated by the method described in Skerget et al. (2005) where Folin-Ciocalteu

Table 1. Thrombolytic (in terms of % of clot lysis) and cytotoxic activities of *Ficus erecta* extracts.

Sample	Cytotoxic activity LC_{50} (µg/ml)	Thrombolytic activity (% of lysis)
ME	1.50±0.06	17.34±0.56
CHP	1.43±0.035	22.51±1.0
PEP	1.20±0.028	12.23±0.08
CTP	1.29±0.012	27.53±0.41
AQP	1.99±0.07	26.38±1.03
VS	0.45±0.003	-
SK	-	65.16±0.064
Blank	-	3.13±0.021

The average values of three calculations are presented as mean ± S.D. (standard deviation); VS = Vincristine sulphate; SK = Streptokinase; ME = Methanolic extract; PEP = Pet-ether soluble partition; CTP = carbon tetrachloride soluble partition; CHP= chloroform soluble partition; AQP = aqueous soluble partition of the methanolic extract of *F. erecta.*

reagent and gallic acid is used as oxidizing agent and standard, respectively (Majhenic et al., 2007). 2.5 ml of Folin-Ciocalteu reagent (10 times diluted with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution were added with 0.5 ml of extract solution (concentrated 2 mg/ml) and was incubated at room temperature for 20 min. After incubation, the absorbance was measured by UV-spectrophotometer at 760 nm. With the help of absorbance & standard curve (having equation of y=0.016x+0.021 and R^2=0.998) which was prepared by putting absorbances at different concentrations of gallic acid solution against the different concentrations, the total phenolic content of the extract was calculated.

Free radical scavenging activity evaluation

The free radical scavenging activities of the plant extractives on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method described by Brand-Williams et al., 1995. 2.0 ml of methanol solution of the extract at different concentrations (ranging from 500 µg/ml to 0.977 µg/ml) were mixed with 3.0 ml of a DPPH methanol solution (20µg/ml) & incubated for 30 minutes in dark. The absorbance was measured by UV spectrophotometer at 517 nm using methanol as blank. Inhibition of free radical DPPH in percent (I %) was calculated as follows:

$$I\ (\%) = (1 - A_{sample}/A_{blank}) \times 100$$

Where, A_{blank} is the absorbance of the control reaction (containing all reagents except the test material). By plotting percentage inhibition of free radicals at different concentration versus concentration of the samples, a regression equation was established and the IC_{50} values (concentration required to scavenge 50% of free radicals) were measured. Here, tert-butylated-1-hydroxytoluene (BHT) and ascorbic acid (ASA) were used as positive controls.

Cytotoxic activity evaluation

To evaluate cytotoxicity of the plant material, the brine shrimp lethality bioassay developed by Meyer et al. (1982) was used (Mclaughlin et al., 1998 and Persoone et al., 1980). To get stock solutions, all the test samples with concentrations ranging from 400 to 0.781 µg/ml were taken and dissolved in 200 µl of dimethyl sulfoxide (DMSO). Then, 5 ml of simulated seawater 10 shrimp nauplii and 100 µl DMSO were added to each test tube. The next day, the test tubes were inspected to count the number of survivors in order to determine the percent (%) mortality for each dilution. By plotting concentration of the samples versus percent (%) mortality, a regression equation was established and LC_{50} values (concentration required to be lethal for 50% of *Artemia salina*) were computed from the regression equation. Here, as positive control, vincristine sulphate was used.

Statistical analysis

Data obtained by analyzing three repetitions of each sample were used for statistical analysis and the values are expressed as mean ± SD. By using the correlation and regression program, analysis of interrelation between total phenolic content and free radical scavenging activity was coducted.

RESULTS AND DISCUSSION

Results of the thrombolytic activity of the extractives of *F. erecta* fruits are presented in Table 1. The carbon tetrachloride soluble partition (CTP) of *F. erecta* exhibited highest thrombolytic activity (27.53±0.41 followed by the aqueous soluble partition) (26.38±1.03%).

The highest cytotoxic activity was obtained with the pet ether soluble partition (PEP) which exhibited the lowest LC_{50} (1.20±0.028 µg /ml) value whereas standard Vincristine sulphate (VS) demonstrated LC_{50} value of 0.45±0.003 µg /ml (Table 1).

The amount of total phenolic content ranges from 1.82±0.025 mg of GAE/100 g of extractives to 43.69±1.4 mg of GAE/100 gm of extractives of *F. erecta* fruits. The highest phenolic content (43.69±1.4 mg of GAE/100 g of extractives) was demonstrated by petroleum ether soluble partition (PEP) (Table 2).

The petroleum ether soluble partition (PEP) revealed maximum free radical scavenging activity (IC_{50} = 7.35±0.08 µg/ml) as compared to ascorbic acid (IC_{50} = 5.80±0.22 µg/ml) in the free radical scavenging assay. This notable free radical scavenging may be interrelated

Table 2. The total phenolic content and free radical scavenging activity of *Ficus erecta*.

Sample	Total phenolic content (mg of GAE/100 g of dried extract)	Free radical scavenging activity (IC_{50} µg/ml)
ME	1.82±0.025	57.35±1.2
CHP	6.82±0.87	35.59±0.91
PEP	43.69±1.4	7.35±0.08
CTP	22.32±0.08	14.62±0.95
AQP	8.81±0.34	30.62±0.92
BHT	-	27.50±0.69
ASA	-	5.80±0.22

The average values of three calculations are presented as mean ± S.D. (standard deviation); BHT = butylated hydroxytoluene; ASA = Ascorbic acid; ME = Methanolic extract; PEP = Pet-ether soluble partition; CTP = Carbon tetrachloride soluble partition; CHP= Chloroform soluble partition; AQP = Aqueous soluble partition of the methanolic extract of *F. erecta.*

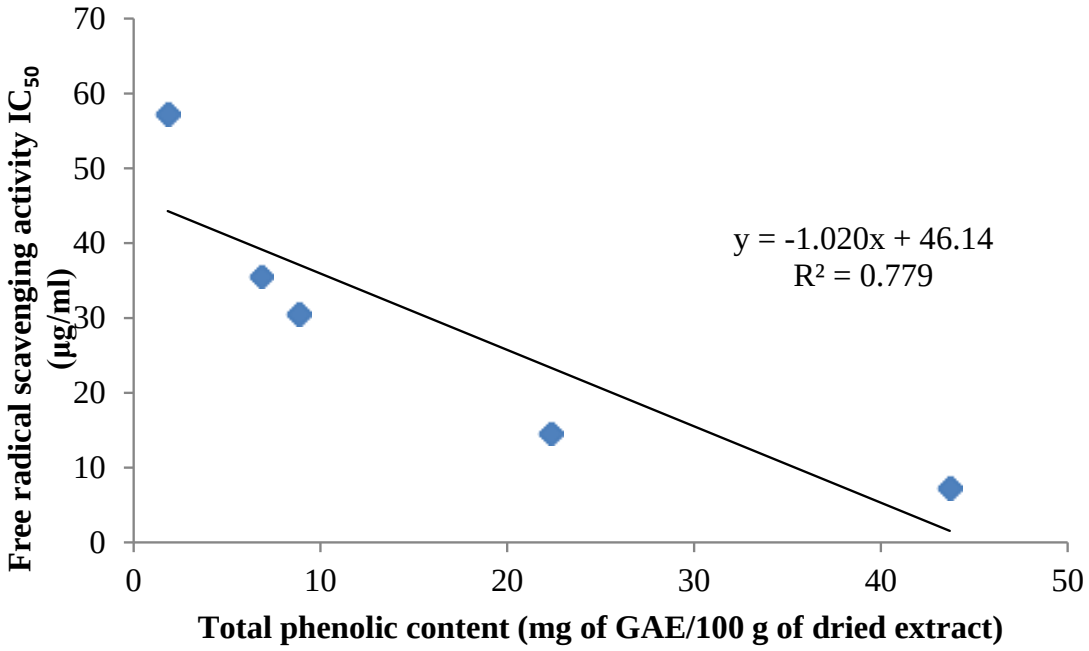

Figure 1. Correlation between the total phenolic content and free radical scavenging activity.

to its high phenolic content (43.69±1.4 mg of GAE/ 100 gm of sample) or due to the presence of various chemical entities which may give synergistic activity. A positive correlation was noticed between total phenolic content and free radical scavenging activity of *F. erecta* which is shown in Figure 1. The correlation coefficient (R^2) was 0.779 which indicated a positive relationship between the total phenolics and free radical scavenging activity. This result implies that about 78% of the free radical scavenging activity of the plant material may result from the contribution of the phenolic compounds (Hajimahmoodi et al., 2008).

From the present analysis, it can be concluded that the extracts of *F. erecta* exhibited strong cytotoxic and antioxidant property and moderate thrombolytic activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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