World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

STUDY ON THE PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF THE AERIAL PARTS OF *FICUS HISPIDA* LINN

Most. Naoshia Tasnin, Ariful Islam, Md. Dobirul Islam, Md. Monirul Islam, Md. Matiar Rahman, MD. Ismail Hossain and Mohammad Amirul Islam*

Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.

***Corresponding Author: Mohammad Amirul Islam**
Department of Biochemistry and Molecular Biology, University of Rajshahi, Rajshahi-6205, Bangladesh.

Article Received on 13/08/2018 **Article Revised on 03/09/2018** **Article Accepted on 24/09/2018**

ABSTRACT

Ficus hispida Linn. (*F. hispida*) is a small but well distributed species of tropical fig tree. Although it is used in traditional medicine for the treatment of many diseases, there is a lack of systemic reports on the phytochemical and antioxidant properties of the aerial parts of that plant. This study was undertaken to investigate the phytochemical and antioxidant properties of *F. hispida* fruits, leaves and bark (aerial parts) using four different solvents (methanol, ethanol, chloroform and n-hexane). Qualitative and quantitative analyses were done for the presence and content of phytochemicals. Six different *in vitro* assay models were used to investigate the antioxidant activity. Phytochemical screening confirmed the presence of alkaloids, flavonoids, flavonols, resins, saponins, proanthocyanidins, glycosides, steroids and tannins. Quantitative analysis demonstrated the total content of polyphenols such as phenolics, flavonoids, flavonols and proanthocyanidins in those extracts. Among all extracts, ethanol extract of *F. hispida* bark showed highest activity in total antioxidant capacity assay, whereas the methanol extract of *F. hispida* bark exhibited maximum activity in ferric reducing antioxidant power assay. In DPPH and Superoxide radical scavenging assays, ethanol extract of *F. hispida* bark showed highest scavenging activity among all extracts with the IC_{50} values of 41.56±2.68 µg/ml and 77.83±4.35 µg/ml respectively. In ABTS scavenging assay, methanol extract of *F. hispida* fruits exhibited maximum activity with IC_{50} value of 50.79±3.67 µg/ml and in Nitric Oxide radical scavenging assay, ethanol extract of *F. hispida* leaves exhibited the highest activity with IC_{50} value of 117.73±2.23 µg/ml.

KEYWORD: Phytochemicals, Antioxidants, *Ficus hispida*, Aerial parts, ABTS, DPPH.

INTRODUCTION

Imbalance between formation and neutralization of Reactive Oxygen Species (ROS) can damage nucleic acids, proteins and lipids and can initiate various chronic diseases like atherosclerosis, cancer, diabetes, cardiovascular disease, ageing and inflammatory diseases.[1,2] Antioxidants help the organism to scavenge free radicals as well as to delay or prevent oxidative stress, thus prevents various degenerative diseases such as cardiovascular diseases, cancers, Alzheimer's disease, neurodegenerative diseases and inflammatory diseases.[1,3] Biological systems have antioxidant defense mechanisms such as catalase, superoxide dismutase, peroxidase glutathione system, etc., that protects against oxidative damages and repair enzymes to remove damaged molecules. These defense mechanisms often fails; hence dietary intake of antioxidant is required.[4] Now a days, synthetic anti-oxidants are used in the food industry, which may be responsible for liver damage and carcinogenesis.[5,6,7,8] Therefore, scientists are interested to find out anti-oxidants from natural sources. Medicinal plants play important role in disease prevention or control has been attributed to their constituents such as vitamins, terpenoids, phenolic acids, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids which are rich in anti-oxidant activity.[9] *F. hispida* (family: Moraceae) is a small but well distributed species of tropical fig tree. It is found everywhere in Bangladesh and also in many parts of Asia and as far southeast as Australia. In Bangladesh it is known as Kakdumur, Khoksha-dumur, Dhungri, Thoska, etc.[10,11] Almost all parts of this plant are used as a folklore remedy for the treatment of various ailments.[12] In Ayurveda *F. hispida* roots are used for the treatment of leukoderma and for the checking intrinsic haemorrhage. Fruits of *F. hispida* are used in the treatment of leukoderma. Its leaves are also used in the treatment of cough and asthma. In Unani system of medicine, *F. hispida* roots and barks are used as blood purifier and drastic purgative. The latex of *F. hispida* is used in ringworm and paste of ripe fruits is used for goiter.[13] *F. hispida* leaves have neuroprotective effect, antidiarrheal activity, hepatoprotective activity, antineoplastic activity, hypolipidemic activity.[14,15,16,17,18]

Its fruits have antimicrobial activity and its roots have wound healing and antiulcer activities.[19,20,21] A few study was done on its fruits, moreover comparative study on its aerial parts is yet to be investigated. Therefore, the present study investigated the phytochemical and antioxidant properties of the aerial parts of *F. hispida*.

MATERIALS AND METHOD

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), potassium ferricyanide, potassium acetate, phosphate buffer, catechin (CA), ferrous ammonium sulphate, butylated hydroxytoluene (BHT), gallic acid (GA), ascorbic acid (AA), $AlCl_3$, Trichloro acetic acid (TCA), sodium phosphate, ammonium molybdate, tannic acid, quercetin (QU), EDTA, thiobarbituric acid (TBA), Methanol, ethanol, chloroform, n-hexane, hydrochloric acids, sulfuric acid, acetyl acetone and $FeCl_3$ were purchased from Sigma Chemical Co.(St.Louis,MO,USA); vanillin was obtained from BDH; Folin-Ciocalteus's phenol reagent (FCR) and sodium carbonate were obtained from Merck (Damstadt, Germany).

Collection of plant material and authentication

Mature *F. hispida* fruits, leaves and bark (aerial parts) were collected from the local area of Rajshahi (north-western part of Bangladesh) and authenticated by the Department of Botany, University of Rajshahi, Bangladesh.

Preparation of extract

The fruits, leaves and bark were first washed with water to remove adhering dirt. Then the fruits and bark were chopped into small pieces. After that the fruits, leaves and bark were shed dried. After complete drying, the entire portions were ground into coarse powder by a grinding machine and stored in an airtight container for further use. Four different solvents namely ethanol, methanol, chloroform and n-hexane were used for extraction. For each solvent about 80 g of the powdered material was taken in separate clean, round bottomed glass bottle and soaked in 400 ml of solvent. The container with its content was sealed by cotton plug and aluminum foil and kept for a period of 15 days accompanying occasional shaking and stirring. The resulting extracts were filtered through Whatman No. 1 filter paper. Afterwards, the solvents were evaporated under reduced pressure at the 40°C using rotary evaporator. Finally, the residues were kept in small sterile bottles under refrigerated conditions until used. Total 12 extracts were prepared from *F. hispida* fruits, leaves and bark (aerial parts), namely: methanol extract *of F. hispida* fruits (MF), ethanol extract of *F. hispida* fruits (EF), chloroform extract of *F. hispida* fruits (CF), n- hexane extract of *F. hispida* fruits (NF), methanol extract of *F. hispida* leaves (ML), ethanol extract of *F. hispida* leaves (EL), chloroform extract of *F. hispida* leaves (CL), n-hexane extract of *F. hispida* leaves (NL), methanol extract of *F. hispida* bark (MB), ethanol extract of *F. hispida* bark (EB), chloroform extract of *F. hispida* bark (CB) and n- hexane extract of *F. hispida* bark (NB).

PHYTOCHEMICAL ANALYSIS

Phytochemical screening of the extracts

Qualitative analysis of phytochemicals such as alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resins, saponins, steroids and tannins was carried out by dissolving samples in specific reagents using standard methods.[22,23]

Determination of total phenolics content

Folin-Ciocalteu method[24] was used to measure the total phenolic contents of each extract. Briefly, 2.25 ml of Folin–Ciocalteu reagent diluted (1:10) in distilled water was mixed with 300 µl of extract and kept for 5 minutes at room temperature. 2.25 ml of sodium carbonate (60 g/l) solution was then added to the mixture and the absorbance was taken at 725 nm after 90 minutes of incubation at room temperature. As standard, Gallic acid (GA) was used and total phenolic content of each extract was calculated as Gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Determination of total flavonoids content

Using previously described method,[24] total flavonoid contents were measured. In short, 2.25 ml of distilled water was mixed with 0.5 ml of extract in a test tube followed by the addition of 0.15 ml of 5% $NaNO_2$ solution. Then test tubes were kept at room temperature for 6 min. After that, 0.3 ml of a 10% $AlCl_3.6H_2O$ solution was added and allowed to stand for 5 min before the addition of 1.0 ml of 1 M NaOH. Then the mixture was vortexed and absorbance was measured immediately at 510 nm. As standard Catechin (CA) was used and results were expressed as catechin equivalents per gram of dry extract (mg CAE/g DW).

Determination of total flavonols content

Total flavonols in the extracts were estimated using the method of Kumaran and Karunakaran.[25] 2.0 ml of extract/standard was taken in the test tube to which 2.0 ml of 2% $AlCl_3$ (dissolved in ethanol) and 3.0 ml (50 g/l) sodium acetate solutions was added. The absorbance measured at 440 nm after 2.5 hr at 20°C. Total flavonols content was expressed in terms of quercetin equivalent, mg of QUE/g of sample dry extract.

Determination of total proanthocyanidins content

Total proanthocyanidins content was determined using the protocol reported by Sun et al.[26] Briefly, 0.5 ml of extract/standard solution was mixed with 3 ml of 4% vanillin in methanol solution, followed by addition of 1.5 ml hydrochloric acid and then the mixture was kept for 15 minutes at room temperature. Then the absorbance was measured at 500 nm. Catechin was used as standard and total proanthocyanidins content was expressed in terms of catechin equivalent, mg of CAE/g of dry extract.

Evaluation of Antioxidant Activity

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) of plant extract was determined by the method of Prieto et al.[27] with some modifications. In short, 0.5 ml of extract at different concentrations was mixed with reaction mixture (3 ml) containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate, and incubated at 95°C for 10 min to complete the reaction. Then spectrophotometric reading was taken at 695 nm against blank after cooling at room temperature. A graph of Catechin (standard) at various concentrations was also constructed in a similar manner for comparison.

Determination of ferric reducing antioxidant capacity

The reducing capacity was evaluated following the method of Oyaizu (1986) with some modification.[28,29] 250 µl of samples/standard at different concentrations was mixed with 1.75 ml of 0.2 M phosphate buffer (pH 6.6) and 1 ml of potassium ferricyanide (1%). Then the mixture was incubated at 50°C for 20 min followed by the addition of 1 ml of TCA (10%). 1 ml from the incubation mixture was mixed in a test tube with 1 ml of distilled water and 0.2 ml of ferric chloride (0.1%). The absorbance of that resulting solution was measured at 700 nm after 10 min. An increased absorbance of the reaction mixture indicates increased reducing power.[30] A standard using ascorbic acid (AA) at various concentrations was also made in a similar manner for comparison.

DPPH free radical scavenging assay

DPPH free radical scavenging capacity of plant extract was determined as previously described methods[31,32] with a little modification. Shortly, 0.5 ml of sample was mixed with 3.5 ml of 0.2 mM methanolic solution of DPPH free radical and the absorbance was taken at 517 nm after incubation of 30 minutes at room temperature. AA and Butylated hydroxytoluene (BHT) were used as positive control. Radical scavenging activity was calculated by the following formula:

% Scavenging Activity = (Acontrol- Asample /Acontrol) × 100 Where, Acontrol = Absorbance of control, Asample = Absorbance of sample.

Then percentage of DPPH radical scavenging activity was plotted against concentration, and from the graph IC_{50} was calculated.

Determination of ABTS radical scavenging activity

The antioxidant capacity of plant extract was determined in terms of ABTS radical scavenging activity following the method previously described.[33] ABTS radical was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate solution and the mixture was left in the dark at room temperature for 12–16 hrs before use. Solution of ABTS radical (stable for 2 days) was diluted with water to obtain an absorbance of 0.70±0.02 at 734 nm. Then ABTS radical solution (3 ml) was added to 1 ml of the test sample with various concentrations and mixed vigorously. After 6 min, the absorbance was measured at 734 nm. AA and BHT were used as positive control. ABTS radical scavenging activity of the samples was expressed as:

% Scavenging Activity = [(Acontrol– Asample) /Acontrol)]×100

Where, Acontrol is the absorbance of the blank control (ABTS radical solution without test sample) and Asample is the absorbance of the test sample.

Determination of superoxide scavenging activity

Superoxide scavenging activity was determined using the NBT (nitrobluetetrazolium reagent) method previously described by Sabu et al.[34] (with some modification). Test solution of extracts (20–300 µg/ml) was taken in a test tube, after that 1 ml of (5 mM) sodium carbonate, 0.4 ml of (0.24 mM) NBT and 0.2 ml of 0.1 mM EDTA solutions were added to the test tube and absorbance was taken at 560 nm immediately. About 0.4 ml (1 mM) of hydroxylamine hydrochloride was added to initiate the reaction and the reaction mixture was incubated at 25^0C for 15 min, and the reduction of NBT was estimated at 560 nm. AA and BHT were used as standards. The % of inhibition was calculated according to the following formula:

$$\% \text{ Scavenging Activity} = [(A_0\text{-}A_1)/A_0] \times 100$$

Where, A_0 is the absorbance of the initial reading of sample/standard and A_1 is the absorbance of final reading.

Nitric oxide (NO) scavenging activity

The method of Garrat et al. was used to determine the nitric oxide radical scavenging activity of plant extracts with some modification.[35] 2 ml of 10 mM sodium nitroprusside prepared in 0.5 ml PBS (pH 7.4) was mixed with 0.5 ml of plant extract at various concentrations. The mixture was incubated for 150 minutes at room temperature. After 150 min incubation, the solution was mixed with 0.5ml of Griess reagent and kept at room temperature for 5 min. After 1 ml of NED (0.1% w/v) was added to the mixture. The mixture was incubated at room temperature for 30 min, followed by the measurement of absorbance at 546nm. AA and BHT were used as positive control. The NO radical-scavenging activity of the samples was expressed as;

$$\% \text{ Scavenging Activity} = [(A_{control} - A_{sample})/A_{control})]\times 100$$

Where, $A_{control}$ is the absorbance of the blank control (NO) radical solution without test sample) and A_{sample} is the absorbance of the test sample.

RESULTS

Phytochemical screening of the extracts

Qualitative phytochemical analysis has revealed the presence of different phytochemicals including alkaloids, carbohydrates, flavonoids, glycosides, triterpenoids, resins, saponins, steroids and tannins in different extracts of *F. hispida* which are enlisted in table 1.

Table 1: The qualitative phytochemical investigation of twelve different extracts of *F. hispida* fruits, leaves and bark.

	Name of the test	Test samples											
		EF	**MF**	**CF**	**NF**	**EL**	**ML**	**CL**	**NL**	**EB**	**MB**	**CB**	**NB**
Alkaloids	Dragendorff's test	–	–	+	–	+	+	–	–	+	+	+	+
	Hager's test	+	–	–	–	–	–	–	–	–	+	–	+
	Wagner test	–	–	–	–	–	–	+	–	–	–	–	–
	Mayer's test	–	+	–	–	–	+	+	–	+	–	+	–
Carbohydrates	Molisch's test	+	+	+	+	+	+	+	+	+	+	+	+
	Benedict's test	+	+	–	–	+	+	–	–	+	+	–	–
	Fehling's test	+	+	–	–	+	+	–	–	+	+	+	–
	Anthrone test	+	–	–	–	–	+	+	+	–	–	–	+
Flavonoids	Shinoda's test	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	Molisch's test	+	+	+	–	+	+	+	+	–	–	+	+
Triterpenoids	Liebermann- Burchard's test	+	+	–	–	+	–	–	–	–	–	–	–
Resins		+	–	–	+	+	+	–	–	+	+	+	+
Saponins		–	–	+	+	+	+	–	–	+	+	+	+
Steroids	Liebermann-Burchard's test	+	–	+	–	+	+	–	+	+	+	+	–
	Salkowski	+	+	–	+	+	+	–	+	–	+	+	+
	reaction												
Tanins		+	–	–	–	+	+	+	–	+	+	+	–

Note: "+" indicates the presence and "–" indicates the absence of the relevant phytoconstituents.

Total phenolics, flavonoids, flavonols and proanthocyanidins contents

Total phenolics, flavonoids, flavonols and proanthocyanidins contents of *F. hispida* fruits, leaves, and bark extracts are shown in table 2, where the relatively higher amount of phenolics, flavonoids, and proanthocyanidins were found in EB of *F. hispida* (153.56±3.22 mg GAE/g DW, 167.79±2.77 mg CAE/g DW and 15.93±0.67 mg CAE/g DW, respectively), but in the EL highest amount of flavonols (49.66±2.16 mg QUE/g DW) was found.

Table 2: Total Phenolics, Flavonoids, Flavonols and Proanthocyanidins contents of *F. hispida* fruits, leaves and bark.

Name of extracts	Phenolics (mg GAE/g DW)	Flavonoids (mg CAE/g DW)	Flavonols (mg QUE/g DW)	Proanthocyanidins (mg CAE/g DW)
EF	82.31±3.89	45.55±2.01	41.95±1.14	4.65±0.29
MF	69.78±1.14	63.44±2.00	38.09±1.08	7.52±0.18
CF	53.20±1.75	66.15±1.70	20.54±0.66	4.96±0.46
NF	21.82±0.66	20.08±0.99	11.77±0.66	13.78±0.73
EL	75.07±1.55	31.19±0.69	49.66±2.16	2.25±0.08
ML	46.86±2.09	44.88±1.20	43.89±0.89	1.22±0.11
CL	51.09±2.31	48.54±0.99	43.35±1.51	10.08±0.27
NL	13.17±0.87	40.54±1.01	34.75±2.12	8.00±0.34
EB	153.56±3.32	167.79±2.77	24.40±1.08	15.93±0.67
MB	147.21±2.64	151.11±1.67	10.36±1.12	13.72±0.40
CB	88.48±1.52	72.93±0.66	44.05±1.55	4.13±0.15
NB	72.43±1.98	29.57±0.39	30.72±0.65	7.83±0.26

Note: Results are expressed as mean ± standard deviation. DW: Dry weight of extract.

Antioxidant Activity of *F. hispida* extracts

The antioxidant activity of *F. hispida* extracts were evaluated by six widely used different assays: Total antioxidant capacity (TAC), Ferrous reducing antioxidant capacity, DPPH, ABTS, Superoxide and Nitric Oxide (NO) scavenging assay. The TAC of different extracts of *F. hispida* and Catechin (standard) is shown in Figure 1. All extracts demonstrated increased

TAC with the increasing concentration of the extracts. All the twelve extracts strongly reduced Mo (VI) to Mo (V) which is comparable to standard, Catechin (CA). Among these twelve extracts, EB exhibited higher activity and CF showed the lowest activity. The Ferric reducing antioxidant capacity of different extracts of *F. hispida* and Ascorbic acid (AA) as standard is shown in Figure 2. Each extract was found to demonstrate higher reducing potential with the increasing concentration of the extracts among them MB showed the highest activity and CF represented the lowest activity. DPPH, ABTS, Superoxide and Nitric Oxide (NO) scavenging activity of twelve extracts of the aerial parts of *F. hispida* and standards were determined in a concentration dependent manner and are presented in Table 3. It is evident from Table 3 that the extracts reduced these radicals more with increasing concentrations. Lower values of IC50 indicated stronger scavenging capacity. EB showed maximum scavenging activity among all twelve extracts in DPPH and Superoxide radical scavenging assays with IC50 values 41.56±2.68 µg/ml and 77.83±4.35 µg/ml respectively, where MF represented maximum scavenging activity in ABTS radical scavenging assay with IC50 value 50.79±3.67 µg/ml and EL exhibited highest scavenging activity in Nitric oxide radical scavenging assay with IC50 value 117.73±2.23 µg/ml. Strong correlation ($p \leq 0.05$) was also found between the phenolic content and the radical scavenging activity of all extracts which are shown in Table 4.

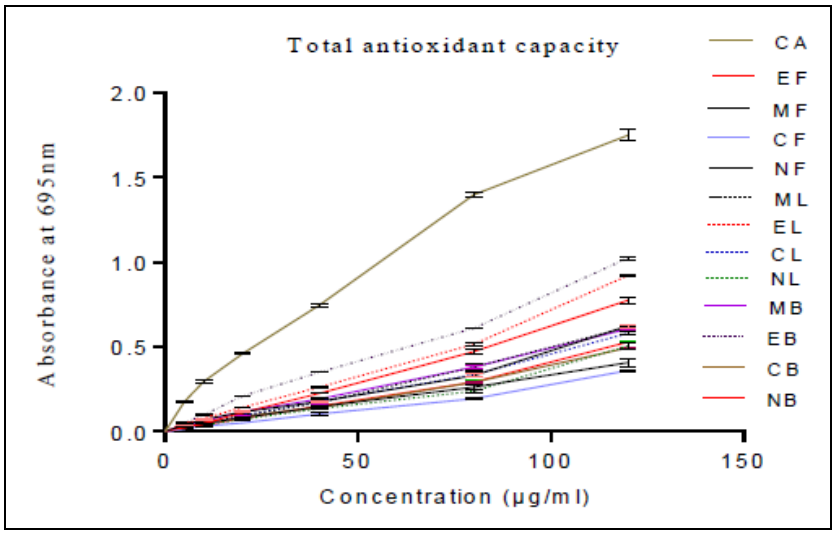

Figure 1: Total antioxidant capacity of different extracts of *F. hispida*.

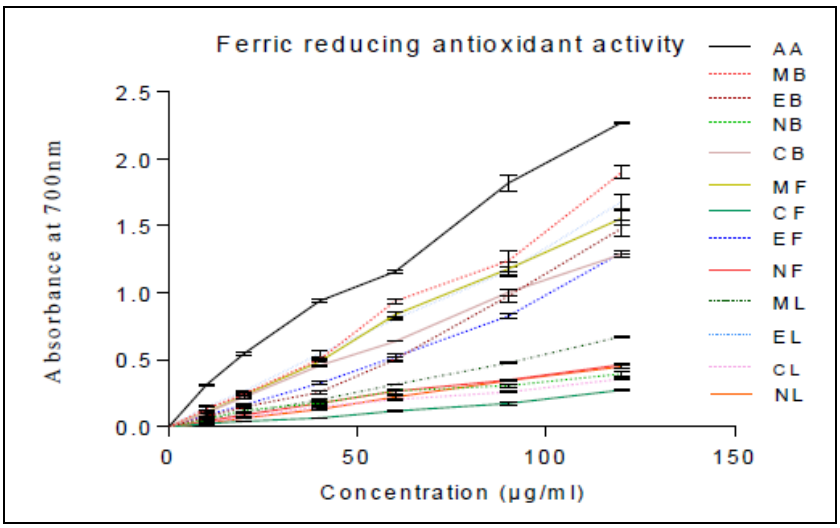

Figure 2: Ferric reducing antioxidant capacity of different extracts of *F. hispida*.

Table 3: IC_{50} values of *F. hispida* fruits, leaves and bark extracts in different assays.

Name of extracts		IC_{50} (µg/ml)			
		DPPH	ABTS	Superoxide	Nitric oxide
EF		71.90±3.66	55.06±4.59	95.68±2.15	237.63±2.82
MF		97.33±4.16	50.79±3.67	78.10±3.90	198.02±3.77
CF		179.1±4.88	266.20±4.21	361.82±3.32	341.37±3.10
NF		270.3±2.82	255.62±3.68	524.36±5.04	533.95±4.33
EL		50.33±2.59	83.35±1.98	105.21±4.40	117.73±2.23
ML		79.10±3.51	103.51±3.58	154.17±3.49	168.98±4.66
CL		328.60±5.78	339.91±6.26	455.36 ± 3.49	818.53±2.07
NL		414.61±4.46	474.40±3.01	593.29 ± 3.21	1027.41±8.93
EB		41.56±2.68	72.22±4.34	77.83±4.35	159.49±6.89
MB		64.23±2.52	92.22±5.07	143.6±4.30	230.82±3.69
CB		123.50±3.17	168.30±3.94	281.10±4.04	580.17±3.26
NB		355.50±5.64	328.32±5.15	856.43±7.12	836.35 ± 5.75
Standard	AA	20.07±1.36	31.41±2.97	58.10±2.23	79.07 ± 2.45
	BHT	35.24±1.86	46.73±2.37	71.59±1.96	98.47±2.73

Note: Results were expressed as mean ± standard deviation (n=3). AA: Ascorbic Acid, BHT: Butylated hydroxytoluene.

Table 4: Correlations between the antioxidative activities and total phenolic contents of the different extracts of the aerial parts *F. hispida*.

Assay	Correlation coefficient (R^2)											
	EF	MF	CF	NF	EL	ML	CL	NL	EB	MB	CB	NB
DPPH	0.97	0.94	0.86	0.9	0.95	0.93	0.89	0.91	0.84	0.92	0.87	0.85
ABTS	0.79	0.81	0.87	0.85	0.86	0.9	0.91	0.92	0.85	0.82	0.85	0.86
Super - oxide	0.87	0.83	0.91	0.96	0.84	0.87	0.87	0.89	0.78	0.84	0.86	0.91
NO	0.83	0.85	0.86	0.85	0.82	0.83	0.93	0.93	0.79	0.86	0.91	0.92

DISCUSSION

In the present study, we have used four different solvents to prepare extracts from the aerial parts of *F. hispida* to detect the presence of alkaloids, flavonoids, carbohydrates, triterpenoids, glycosides, saponins, resins and tannins as well as to evaluate the total phenolics, flavonoids, flavonols and proanthocyanidins contents and antioxidant activity. The findings of our current study varied from extract to extract. The differences in polyphenol contents and biological activities of plant extract depend on the type of solvent used.[36,37]

Phytochemicals are the chemical constituents in plants with prominent biological activities such as anti-inflammatory, antioxidant, anticancer and antimicrobial properties. Phenolics and flavonoids are the common antioxidants known in plants.[38] Moreover, Proanthocyanidins play preventive role in various diseases, like atherosclerosis, large bowel cancer, cataracts, gastric ulcer and diabetes. Flavonols also have significant cardiovascular health benefits.[39,40] The present study showed that each extracts of *F. hispida* contain a significant amount of total phenolics, flavonoids, flavonols and proanthocyanidins.

Now a days, polyphenols have attracted particular attention due to their ability to reduce free radical induced tissue injury. In this present study, we have used six in vitro assay models to evaluate the antioxidant activity of *F. hispida* extracts. TAC is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acidic pH. TAC of the phosphomolybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity. In ferric reducing antioxidant capacity/power (FRAP) assay, ferric-ferricyanide complex is reduced to the ferrous form due to the presence of antioxidants in the plant extracts. Therefore, the concentration of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm[41]. In TAC assay EB showed highest antioxidant activity than other extracts, whereas MB represented maximum activity in FRAP assay. DPPH is a stable free radical due to the delocalization of the spare electron on the whole molecule. It is widely used for measuring the free-radical scavenging activity of plant extracts and pure compounds, where stable DPPH free-radical is reduced by antioxidants leading to the development of a yellowish colored compound.[42] The ABTS radical scavenging assay is also commonly used to measure the radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plants extracts.[43] Active free radicals such as hydroxyl radical, hydrogen peroxide and singlet oxygen can be generated from superoxide anions. Although hydrogen peroxide is not so much toxic to cell, but it may give rise to hydroxyl radical which have the ability to interact with DNA directly and therefore contribute to cancer development,

ageing and cytotoxicity. Injurious NO is also generated in biological tissues by specific nitric oxide synthases.[2,44] In our present study, EB showed maximum radical scavenging activity in DPPH and Superoxide scavenging assay, EL represented highest radical scavenging activity in Nitric Oxide scavenging assay and MF revealed maximum radical scavenging activity in ABTS scavenging assay compared to that of standard reference compounds, ascorbic acid and butylhydroxytoluene. All other extracts also represented moderate radical scavenging activity compared to the standards in all these assays.

CONCLUSION

This study concluded that *F. hispida* is a promising source of therapeutically important phytochemicals and antioxidants. It can play an important role as a therapeutic agent in the protection against oxidative stress. The present study also recommended that methanol and ethanol are preferable solvents for the extraction of polyphenols from the aerial parts of *F. hispida*, ethanol is better for the extraction and bark of *F. hispida* contains much more polyphenols than its fruits and leaves. Further investigation is required to identify and characterize the active compounds presents in the aerial parts of *F. hispida*.

ACKNOWLEDGEMENT

The authors are grateful to all the members of the Laboratory of Clinical Biochemistry and Nutritional Sciences for their constant support throughout this research and Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh for supplying necessary materials.

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