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ANTIOXIDANT ACTIVITY OF *FICUS RACEMOSA* PLANT EXTRACTS OF LEAVES AND BARKS

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ABSTRACT

Present investigation is an attempt to evaluate antioxidants potential of leaves and stem bark of Ficus racemosa (Moraceae), an important medicinal plant in the Indian system of medicine. Research on antioxidant activity is an important topic in the medical sector as well as in the food industry due to the fact that, antioxidants play a major part in inhibiting and scavenging free radicals, thus providing protection to human against infections and degenerative diseases. It reduces cell damages caused by free-radical which are responsible for various ailments like ageing, cancer, coronary heart disease, diabetes mellitus, neurodegenerative disorders, atherosclerosis, cataracts and inflammation. Methanolic extract was prepared from the leaf and stem bark of Ficus racemosa. Their total phenolics and free radical scavenging capacity was determined using folin-ciocalteu reagent (FCR) assay and DPPD-radical scavenging assay respectively. Stem bark and leaves of F. racemosa contains 239.73 and 229.37 mg of GAE / gm of dried extract of phenolics respectively. Current studies also show that Ficus racemosa barks contain a high antioxidant activity. IC50 value of bark extract was found to be 19µg/ml which is even better that of the standard BHT. Therefore, present studies suggest that F. racemosa bark has a huge potential in terms of commercial use as source of antioxidant.

KEY WORDS

Ficus racemosa, plant extract, phenolic content, antioxidant.

INTRODUCTION

India has an ancient heritage of traditional medicine. The Materia Medica of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various systems including Ayurveda, Siddha, Unani and Homoeopathy. The evaluation of these drugs is primarily based on phytochemical, pharmacological and allied approaches including various instrumental techniques such as chromatography, microscopy and others. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential. In this regard, one such plant is *Ficus racemosa* Linn.syn. *Ficus glomerata* Roxb. (Family: Moraceae). The plant is a large deciduous tree distributed all over India from outer Himalayan ranges, Punjab, Khasia mountain, Chota Nagpur, Bihar, Orissa, West Bengal, Rajasthan, Deccan and common in South India [1]. It is the member of the four sacred trees Nalpamara (Ksirivrksas) meant to be planted around the home and temples. It is found throughout the year, grows in evergreen forests, moist localities and bank of streams, deciduous forests, to the elevation of 1800m above sea level, often cultivated in villages for shade and its edible fruits [2-5]. It is commonly known as gular fig, Cluster fig in English, Gular in Hindi and as Udumbara in Sanskrit [6-8]. The

tree is up to 18m high, leaves ovate, ovate-lanceolate or elliptic, sub acute, entire and petiolate. Leaves are shed by December and replenished by January and April, when the tree becomes bare for a short period. Figs subglobose or pyriform, red when ripe, borne in large clusters, on short, leafless branches emerging from the trunk and the main branches [1, 9]. The tree is without aerial roots unlike its many family members. It naturally comes up in wasteland and forests in subtropical climate. It is seen dwelling in areas up to 1200m altitude on hilltop. This requires well-drained medium to heavy soils for its successful cultivation and comes up in all kinds of soil except in water logged and clay soil [8].

MATERIALS AND METHODS

Chemicals

2, 2-Diphenyl-2-picryl hydrazyl (DPPH), Folinciocalteu reagent (FCR) was obtained from Sigma– Aldrich (St. Louis, USA.) Butylated hydroxy toluene (BHT), methanol was purchased from Merck (Germany) and gallic acid was procured from Wako pure chemicals Ltd., Japan. All other chemicals used in the current paper were of analytical grade.

Collection of samples and preparation of extracts

Leaves (FL) and stem barks (FB) of *Ficus racemosa* plant were collected from different places of Rajshahi in May, 2011. Upon arrival at the laboratory, samples were washed thoroughly under running tap water and then dried at 45°C for 48 h. The dried samples were stored in sealed polyethylene bags with silica gel. Dried leaf and bark samples were ground into coarse powder using a grinding machine. 100 gm of powdered plant materials (Leaves and stem barks) were taken in 1L conical flasks and allowed for soaking in 500 ml of methanol for each sample. The conical flasks with its contents were then sealed and kept on orbital shaker for continuous shaking at 150 rpm for 2 days. Sonication was also performed with the help of an Ultrasound Bath for breaking the cell walls completely. Followed by sonication the conical flasks were kept again on orbital shaker for 1 day. The mixtures were then filtered through Whatman No.1 filter paper and solvents were completely evaporated using a rotary evaporator under controlled temperature (45°C).

Antioxidant activity test

Total phenolic content determination

Total phenolic content of methanolic extracts of different parts, including FL (*F. racemosa* leaf), FB (*F. racemosa* bark) were determined by the method reported by Chang et al. 2002; involving folin-ciocalteu reagent (FCR) as oxidizing agent and gallic (GA) acid as standard. The results were expressed as mg of GAE/gm of dried extractives.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

The free radical scavenging capacity of the extracts was determined using DPPH (Choi et al, 2000; Desmarchelier et al. 1997). DPPH solution (0.004% w/v) was prepared in 95% methanol. 1.0 ml of methanol solution of different extracts and standard at different concentrations (25 µg/ml, 50 µg/ml, 100µg/ml, 150 µg/ml and 200 µg/ml) were taken in test tubes. 1.5 ml of methanol solution of DPPH was added into each of the test tubes. The test tubes were then incubated at RT (room temperature) for 30 minutes in dark place to complete the reaction. Then the absorbance of the solutions were measured at 519 nm using a spectrophotometer against blank. BHT was used as a reference standard and dissolve in distilled water to make the stock solution with the same concentration. A typical blank solution contained all reagents except plant extract or standard solution also taken as control. The percentage (%) inhibition activity was calculated from the following equation

$$\% I = \{(Ao - A1)/Ao\} X 100$$

Where,

A0 is the absorbance of the control, and

A1 is the absorbance of the extract/standard.

Then % inhibitions were plotted against concentration, and IC50 was calculated from the graph.

Evaluation of Repellent Activity of *F. racemosa* extracts

To check whether *F. racemosa* has any insectidal activity, repellent activity test against the stored grain pest *Tribolium castaneum* was carried out. The repellency test used was adopted from the method of McDonald et al. (1970) with some modifications by Talukder & Howse (1994). Half filter paper discs (Whatman No. 40, diameter 90 cm) were prepared and selected doses of all the methanol extract separately applied onto each of the half-disc and allowed to dry out by exposed in the air for 10 minutes. Each treated half-disc was then attached lengthwise, edge-to-edge, to a control half-disc with adhesive tape and placed in a Petri dish (diameter 90 cm). Ten adult insects were released in the middle of each filter-paper circle. Each concentration was tested five times. Insects that settled

on each half of the filter paper disc were counted after 1h and then at hourly intervals for 5h. The average of the counts was converted to percentage repellency (*PR*) using the formula:

PR = 2(C - 50),

Where, C is the percentage of insects on the untreated half of the disc. Positive values expressed repellency and negative values for attractant activity. The values in the recorded data were then calculated for percent repellency.

Evaluation of *In vitro* Antibacterial Activity of *F. racemosa* extract

Five (5) mg of each extracts of *F. racemosa* were added carefully to 5 ml of de-ionized water and dissolved by vortexing the tube. The stock extract solution was prepared as 1µg /µl. The stock of *Bacillus subtilis* and *Enterobacter sp.* strains were taken out of -20° and revived in LB medium. Culture was incubated at 37°C for overnight. These suspensions were used as inoculum**.** Disk diffusion method was used in the current experiment to evaluate the antibacterial activity of FL, FB extracts. The discs (6mm diameters) were made by punching the Whatman No. 1 filter paper with the help of punch machine. These discs were taken into the screw capped tube and sterilized in an autoclave machine at 121°C for 20 minutes to ensure sterilization. The paper discs were soaked with different concentrations (50µg, 100µg, 150µg and 200µg) of each extract with the help of micropipette and keep them at laminar air flow hood for dryness (5-10 minutes). For standard the disc papers were prepared in a similar way using 30µg of Chloramphenical. The disks containing sample as well as the standard were introduced on the upper layer of the seeded agar plate by sterile forceps. After that the plates were incubated overnight at 37°C and diameter of zone of inhibition (cleared area around the disk) was measured.

RESULTS

Determination of Total Phenolics

The total phenolic content of methanolic extracts of different parts of *F. racemosa* was determined by using folin-ciocalteu reagent (FCR) as oxidizing agent and gallic (GA) acid as standard. Absorbance was measured at 750 nm wavelength after 20 minutes of incubation at room temperature. Both leaves and stem bark methanolic extracts showed similar result in terms of total phenolic content. Among the two samples the highest phenolic content was found in *F. racemosa* stem bark which were 239.73 and 229.37 mg of GAE / gm of dried extract of phenolics respectively dried extract at concentration of 200 µg/ml (Fig.1). So, it is obvious that stem bark of *F. racemosa* possessed slightly higher phenolic content than that of le–aves. Result of the total phenolic content determination are tabulated in Table 1 and depicted in Figure 1.

Table 1; Determination of Total Phenolics. Absorbance of GA (standard) at different concentrations and absorbance of FL and FB extracts at 200 µg/ml after treatment with Folin-Ciocalteu reagent. Absorbance of each sample was taken in triplicated indicated as a, b and c.

Samples	Conc. (µg/ml)	Absorbance			Absorbance Mean ± STD	GAE/gm of dried Extract
		A	B	C		
	20	0.293	0.297	0.319	0.305±0.014	--
	40	0.518	0.532	0.541	0.532±0.01159	--
Gallic Acid	80	1.011	1.015	1.003	1.007±0.00611	--
	160	1.778	2.012	1.877	1.893±0.117461	--
	320	3.789	3.593	3.798	3.727±0.115846	--
FL	200	0.803	0.859	0.968	0.878±0.083907	229.73
FB	200	1.088	1.241	1.121	1.152±0.080517	239.37

DPPH (1, 1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

The 1, 1-diphenyl-2-picrylhydrazyl radical (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. With this method, it is possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 519 nm. Resulting from a color change from purple to yellow, the absorbance

decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form, this molecule had an absorbance at 519 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule. 1.0 ml of methanolic extract solution of *F. racemosa* leaf and bark sample was taken for the experiment. Decolorization of DPPH by the effect of the extracts were measured at 517 nm. The results of DPPH radical scavenging assays of butylated hydroxytoluene (BHT) (standard) and plant extracts are tabulated in table 2. Figure 2 showed free radical scavenging activity of the two extractives used in the current paper. At a concentration of 200 µg /ml,the scavenging activity of the *F. racemosa* leaves and bark extracts were found to be 48.54701% and 73.46154% respectively, while at the same concentration, the activity of BHT was 96.35%. Thus, *F. racemosa* stem bark exhibited significant free radical scavenging activity. The IC50 of stem bark of *F. racemosa* was 19 µg/ml.

Table 2: DPPH free radical scavenging activity of methanolic extracts of standard BHT and FL and FB at various concentrations. Absorbance of each sample was taken in triplicated indicated as a, b and c.

Name of Sample	Conc(µg/ml)	% of Scavenging			Mean % of scavenging ±SD	IC_{50} (µg/ml)
		A	B	C		
BHT	25	36.51	36.63	36.71	36.61±0.100664	36
	50	63.73	63.79	63.59	63.70±0.102632	
	100	87.41	87.56	87.49	87.48±0.075056	
	150	95.39	95.36	95.37	95.37±0.015275	
FL	25	39.91	39.77	39.54	39.74±0.186815	--
	50	40.03	41.65	39.33	40.33±1.190014	
	100	44.23	44.56	44.19	44.32±0.20306	
	150	48.42	45.77	44.91	46.36±1.82949	
FB	25	61.77	59.63	59.17	60.19±1.387516	19
	50	70.23	69.57	68.31	69.37±0.9755	
	100	74.67	70.43	68.17	71.09±3.299879	
	150	75.47	74.21	73.31	73.38±1.084988	

Fig:1: Standard Curve for Gallic acid

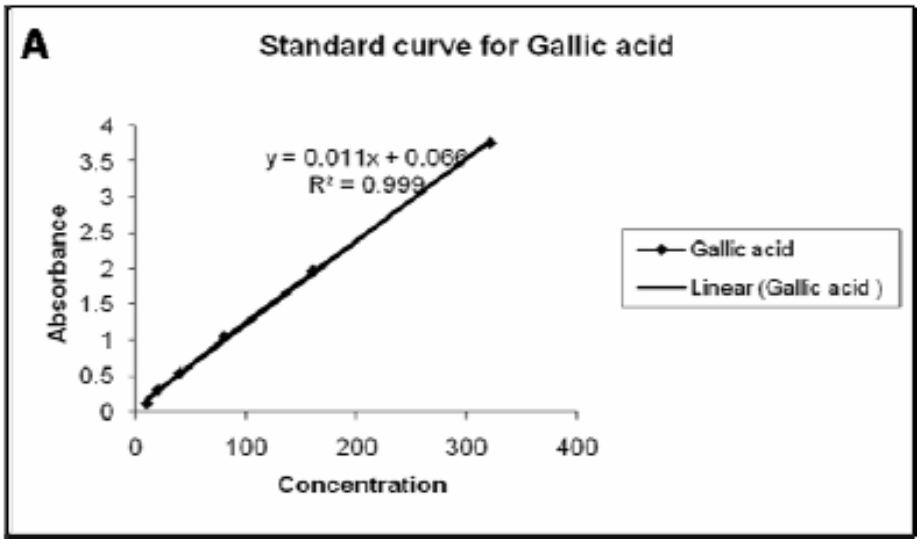

Table 3: Antimicrobial activity of FL and FB at various concentrations

Sample	Sample concentration/dose (µg)	Inhibition zone after 24 hour(mm)		Inhibition zone by Chloramphenicol at 30 µg conc. (mm)
		Bacillus subtilis	*Enterobacter sp.*	
FB	50	4	--	30
	100	8	1.5	
	150	13	3	
	200	15	4	
FL	50	--	--	0
	100	--	--	
	150	--	--	
	200	--	--	

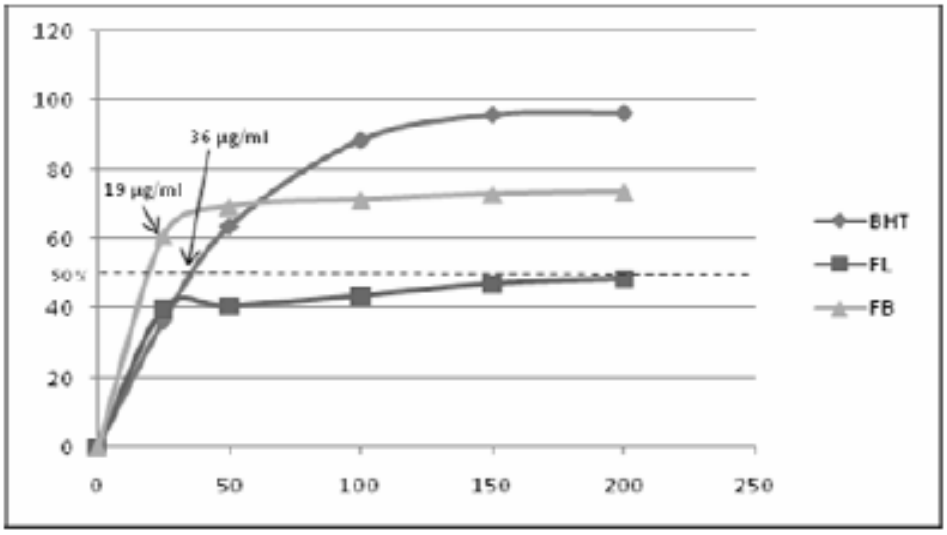

Fig. 2: IC_{50} (µg/ml) values of methanolic extractives of FB, along with BHT as standard for DPPH radical scavenging activity. IC50 value is shown using red dotted line and indicated with arrow.

Repellant activity

Both the leaf and stem bark extracts of *F. racemes'* were tested against *T. castaneum* adults for their repellent activity. However, either of the extracts did not show any significant repellency against the adult beetles.

Estimation of *In vitro* Antibacterial Activity of *F. racemosa*

Antimicrobial activity test of FL and FB was done against two bacterial species: *Bacillus subtilis* and *Enterobacter sp.* in different concentrations. As control, 30 mg/ml chloramphenicol was used. However, not much activity was found in any of the FL and FB samples compared to control. Only high concentration of FB extract showed moderate antimicrobial activity (15mm clearing zone) against *Enterobacter sp.* FL did not show any antimicrobial activity.

DISCUSSION

Antioxidants works as natural healer in repairing oxidative cell damages and thus prevents a lot of physical ailment. A great number of aromatics, medicinal, spices, vegetable, fruits and other plants contain chemical compounds exhibiting antioxidant properties. Various workers also reported high antioxidant activities of different plant extracts using different assaying methods. It was reported that strong DPPH free radical scavenging activity and ferric ions reducing power in sweet potato leaves. Betel leaves had been reported to be more potent than BHT which is similar to our result. It was identified that 10 flavonoid compounds and found three hydrophilic sulfated flavonoids in laksa leaves which exhibited potent antioxidant properties. Betalains responsible for the intense red color of red amaranthus were also powerful scavengers of the DPPH free radical and some of them were found to be stronger than rutin, catechin and

ascorbic acid. It was reported high total flavonoid content in spring onion leaves. At present no clear data is available on the antioxidant content and extent of their activity of antioxidants have a huge potential in industrial and pharmacological application. Results of the current studies show that leave and stem bark of *F. racemosa* contains high level of antioxidant activity. Although, a number of works have been carried out on antioxidant properties from different plant part but data from the methanolic leaf and bark extract is scantly. Results indicate that both leaf and bark extract of *F. racemosa* contain nearly equivalent amount of polyphenol content (239.73 and 229.37 mg of GAE / gm of dried extract respectively). However, bark extract shows significantly higher free radical scavenging activity compared to the leaf extract. IC50 value of bark extract was found to be 19µg/ml. However, the leaves did not show IC50 from the concentration of 25-200 µg/ml. This indicates that *Ficus* leave extractives may require more than 200µg/ml extract for showing IC50. So, it is clear that *F. racemosa* bark sample had higher scavenging activity even that of the standard BHT which is a significant in terms of commercial use of *F. racemosa* bark as source of antioxidant.

CONCLUSION

Currently, any antioxidant containing drugs and cosmetics is not manufactured in our country although we have affluent and diversified flora resources with very rich history of their medicinal use in 'Ayurveda'. Antioxidant can be used to produce medicines and cosmetics so that, the drug and cosmetic industries in our country can have their own herb-based formulation. The measurement of the antioxidant capacity of selected plants is important to quantify the intake of natural antioxidants in a dietary supplement. Additionally, these studies will help in the selection of natural source of antioxidant and in the use of health relevant food industry. Its constituents scavenge free radicals and thus may contribute a protective effect against oxidative damage induced to cellular macromolecules. However, further studies for the isolation and identification of individual bioactive compounds and also *in vivo* studies are needed for understanding their mechanism of actions well.

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