IJPSR (2011), Vol. 2, Issue 4 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 04 January, 2011; received in revised form 12 March, 2011; accepted 22 March, 2011

STUDIES ON ANTIBACTERIAL, CYTOTOXIC AND ANTIOXIDANT PROPERTIES OF THE SEEDS AND LEAVES OF *FICUS RACEMOSA*

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Keywords:

Antibacterial,

Cytotoxicity,

Antioxidant,

Ficus Racemosa

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ABSTRACT

The seeds and leaves of *Ficus racemosa* have shown considerable antioxidant and cytotoxicity properties while their antibacterial activities were found low. The extracts were found to have different levels of antioxidant and cytotoxicity properties in different concentration. For antioxidant activity, here we used free radial scavenging activity of plant extracts against stable DPPH (1, 1-diphenyl 2-picrylhydrazyl) which was determined spectrophotometrically by the method described in Brand William *et al*. (1995). Methanolic extracts of these plants were used. As we know that ascorbic acid is a perfect antioxidant, that's why it was used as a standard reference. The absorbance was taken at 517nm by UV spectrophotometer. The IC_{50} (Inhibitory Concentration) value of standard was 8.287µg/ml. The IC_{50} value of *Ficus racemosa* seeds and leaves were 3.893µg/ml and 14.5µg/ml respectively. For Cytotoxicity, brine shrimp lethality bioassay was used. We used Vincristine sulfate as a standard reference. The LC_{50} value of standard was 0.057µg/ml. The LC_{50} (Lethal Concentration) value of the seeds and leaves were 0.56µg/ml and 0.467µg/ml respectively. The plant has shown significant activities against *Surcina lutea*.

INTRODUCTION: The medicinal plants find application in pharmaceutical, cosmetic, agriculture and food industry. Recently the uses of natural drugs are under review and have been increasing day by day. Traditional use of medicines is recognized as a way to learn about potential future medicines. In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from "Ethnomedical" plant sources; 80% of these compounds were used in the same or related manner as the traditional ethnomedical use [1]. It is becoming clearer that the medicinal herbs have a potential in today's synthetic era as numbers of medicines are becoming ineffective against resistant microorganisms.

Many of the herbs and spices used by humans to season food yield useful medicinal compounds [2, 3]. Since antioxidant, cytotoxicity and antibacterial constituent are an important medicinal substance; recently research is being continued on various plants to derive antioxidant, cytotoxicity and antibacterial ingredients. But, the endeavor in this regard is not adequate enough. The present research is therefore, an effort to give a detailed survey of the literature on its pharmacological properties.

Plant review: The local name of the plant is Cluster and the taxonomy is as follows-

Kingdom- Plantae
Division – Magnoliophyta
Class – Magnoliopsida
Order – Rosales
Family – Moraceae
Genus – *Ficus*
Species – *Ficus racemosa*

Chemical constituents: Leaves contain Tetra triterpene, glauanol acetate, racemosic acid [4]. Bark contains ceryl behenate, lupeol, lupeol acetate, α & β-amyrin, gluanol acetate, β -sitosterol, stigmasterol and ketone [5].

Medicinal Uses: Scientific studies indicate *F. racemosa* to posses various biological effects such as hepatoprotective [6], chemopreventive [7], antidiabetic [8], anti inflammatory [9], antipyretic [10], antitussive [11] and antidiuretic [12] properties. Bark is astringent, and is used in asthma, menorrhagia, bronchitis and bilious affections [13].

MATERIALS AND METHODS:

Collection and Identification of the Plant: The fresh seeds and leaves of the *Ficus racemosa* were collected during the month February 2009 from the area of Puran Dhaka. The National Herbarium taxonomically identified the plant.

Drying and Pulverization: The fresh seeds and leaves of the plant were first washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinding machine and were stored in an airtight container for further use.

Extraction of Plant Material: The powdered 60gm powders were extracted with 3 times methanol of their weight in a flat bottom glass container, through occasional shaking and stirring for 10 days. The extracts were then filtered through filter paper. The filtrates were concentrated at 50^{o}C under reduced pressure to afford a greenish mass of biological investigation**.**

***In Vitro* Antimicrobial screening:** Disc diffusion method was used to determine the antimicrobial activity of the crude extracts [14, 15], against the microbial strains listed in **Table 1**. These were collected as pure cultures from the microbiology lab of Atish Dipankar University of Science and Technology, Bangladesh To get a concentration of 2000µg/ml, dry Methanol extract (4mg) was dissolved in 2ml methanol & carefully dried to evaporate the residual solvent. Here, Kanamycin (30µg/disc) was used as the standard.

Then, for 24 hours, these plates were kept at low temperature (4^{o}C) to allow maximum diffusion of the test materials and Kanamycin. To allow maximum growth of the organisms, these plates were then incubated for 12- 18 hours, at 37^{o}C. The test materials, which possess antimicrobial activity, suppressed the growth of the microorganisms and a clear, distinct zone of inhibition was seen surrounding the discs. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition, expressed in mm.

Antioxidant Activity: The antioxidant activity, which refers to the free radical scavenging activity, of the extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Brand-Williams et al (Use of a free radical method to evaluate antioxidant activity, 1995). 2mg of each of the extracts was dissolved in methanol. Solutions of varying concentration such as 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.625 µg/ml, 7.8125 µg/ml, 3.90625 µg/ml, 1.953125 µg/ml and 0.9765625 µg/ml were obtained by serial dilution technique.

2 ml of a methanol solution of the extract of each concentration was mixed with 3 ml of a DPPH-methanol solution (20µg/ml) and was allowed to stand for 20 minutes for the reaction to occur. The absorbance was determined at 517nm and the corresponding percentages of inhibitions were calculated from these values by using the following equation:

% inhibition
= [1 - (ABS sample / ABS control)] x 100 [ABS = absorbed]

Then % inhibitions were plotted against respective concentrations used and from the graph IC50 was calculated by using tert-butyl-1-hydroxytoluene (BHT), ascorbic acid potential antioxidant, were used as positive control.

Brine shrimp lethality bioassay: Brine shrimp lethality bioassay [5, 15, 16,] technique was applied for the determination of general toxic property of the plant extracts. In this method, in vivo lethality in a simple zoological organism (Brine shrimp nauplii) is used as a favorable monitor for screening and fractional in the discovery of new biotic natural products. 2 mg of each sample was dissolved in specific volume of DMSO (Dimethyl Sulfoxide) to obtain the desired concentration of the prepared solution. For the experiment, 4 mg of each of the petroleum ether, carbon tetrachloride and chloroform soluble fractions were dissolved in DMSO and solutions of varying concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, 1.953125, 0.9765625 µg/ml) were obtained by serial dilution technique using DMSO for each extract. Vincristine sulfate was used as positive control.

RESULTS & DISCUSSION: The outcome found for the antimicrobial, cytotoxic and antioxidant activities of seeds and leaves of *Ficus racemosa* are discussed in detail in this chapter. It was shown that the specimen has shown significant activities for cytotoxic and antioxidant activities while poor activities are found for antimicrobial properties.

Antimicrobial activity: The Methanolic extract of *Ficus racemosa* was tested for antibacterial activities against a number of both gram positive and gram negative bacteria. Standard kanamycin discs (30 mg/disc) were used for the comparison purpose. The result of antimicrobial activity was measured in term of zone of inhibition (mm), have been shown in **Table 1**. The methanolic extracts were concentration used in 500 µgm/disc. From the above data, it is evident that the methanolic extract of *Ficus racemosa* (seed) has the activity against gram positive bacteria of *surcina lutea* and its zone of inhibition was 21 mm. The zone of inhibition for the *Ficus racemosa* (leaves) against both gram positive & gram negative bacteria were 6-10 mm, 6-11 mm, 6-14 mm & 8-14 mm respectively. The

Methanolic extract of *Ficus racemosa* (leaves) has poor activity against gram (+) ve & gram (-) ve bacteria.

TABLE 1: DIAMETER OF THE ZONE OF INHIBITION OF DIFFERENT PLANT EXTRACTS

Name of Bacteria	*Ficus rasemasa seeds*	*Ficus rasemasa leaves*
Gram positive		
Staphylococus aereus	10	8
Bacillus megaterium	9	10
Bacillus subtilis	10	9
Sarcina lutea	21	10
Gram negative		
Salmonella paratyphi	6	6
Salmonella typhi	6	6
Escherichia coli	6	6
Shigella dysenteriae	6	6
Vibrio minicus	6	6
Vibrio parahemolyticus	11	10
Shigella boydii	6	6
Psedomonas aeruginosa	8	14

Cytotoxic activity through Brine Shrimp lethality bioassay: In this method, *in vivo* lethality in a simple zoological organism (Brine shrimp nauplii) is used as a favorable monitor for screening and fractional in the discovery of new biotic natural products. 2 mg of each sample was dissolved in specific volume of DMSO (Dimethyl Sulfoxide) to obtain the desired concentration of the prepared solution. For the experiment, 4 mg of each of the petroleum ether, carbon tetrachloride and chloroform soluble fractions were dissolved in DMSO and solutions of varying concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.8125, 3.90625, 1.953125, 0.9765625 µg/ml) were obtained by serial dilution technique using DMSO for each extract. Vincristine sulfate was used as positive control.

The Methanolic extracts of samples were subjected to brine shrimp lethality bioassay and Vincristine sulfate was used as standard in this investigation. The extracts were found to have different cytotoxic activity. The standard Vincristine sulfate has the LC_{50} value of 0.057 µg/ml. According to **Table 2**, methanol extracts of *Ficus racemosa* (seeds) the LC_{50} value is 0.56 µg/ml which indicating good cytotoxicity properties. **Table 3** indicates that the LC_{50} Value of methanol extract of *Ficus racemosa* (leaves) is 0.467 µg/ml which indicates good cytotoxicity properties.

TABLE 2: LC50VALUE OF METHANOLIC EXTRACT OF *FICUS RACEMOSA* SEEDS

Concentration (C) µg/ml	Log C	No.of Nauplii taken	No.of Nauplii alive	No.of Nauplii dead	% of morality	LC50 µg/ml
500	2.6989	10	0	10	100	**0.56**
250	2.3979	10	1	9	90	
125	2.09691	10	2	8	80	
62.5	1.79588	10	2	8	80	
31.25	1.49485	10	3	7	70	
15.625	1.19382	10	4	6	60	
7.8125	0.89279	10	2	8	80	
3.90625	0.5917	10	5	5	50	
1.953125	0.29073	10	6	4	40	
0.9765625	-0.0103	10	7	3	30	

TABLE 3: LC50 VALUE OF METHANOLIC EXTRACT OF *FICUS RACEMOSA* LEAVES

Concentration (C) µg/ml	Log C	No. of Nauplii taken	No. of Nauplii alive	No. of Nauplii dead	% of mortality	LC50 µg/ml
500	2.6989	10	0	10	100	**0.467**
250	2.3979	10	0	10	100	
125	2.09691	10	2	8	80	
62.5	1.79588	10	2	8	80	
31.25	1.49485	10	3	7	70	
15.625	1.19382	10	4	6	60	
7.8125	0.89279	10	2	8	80	
3.90625	0.5917	10	5	5	50	
1.953125	0.29073	10	6	4	40	
0.9765625	-0.0103	10	7	3	30	

Antioxidant activity: The methanolic extracts of the plant was subjected to free radical scavenging activity by the method of Brand Williams *et al*., (1995) [17] and ascorbic acid was used as standard in this investigation. The antioxidant activity of the plant was measured by free radical scavenging assay with DPPH. IC_{50} value (concentration of sample require to scavenge 50% free radical or to prevent lipid peroxide by 50%) of all the extracts were calculated. IC_{50} value of standard ascorbic acid was 8.287 µg/ml. The methanolic extract of *Ficus racemosa* (seeds) and *Ficus racemosa* (leaves), the IC_{50} value is 3.8938 µg/ml and 14.55 µg/ml in **table 4** and **5**. This indicates significant antioxidant properties.

TABLE 4- IC_{50} VALUES OF *FICUS RACEMOSA* (SEED)

Concentration (µg/ml)	Absorbance of extract	Absorbance of blank	% inhibition	IC50 (µg/ml)
500	0.079	0.410	80.73170732	**3.893**
250	0.120	0.410	70.73170732	
125	0.169	0.410	58.7804878	
62.5	0.177	0.410	56.82926829	
31.25	0.178	0.410	56.58536585	
15.625	0.186	0.410	54.63414634	
7.8125	0.198	0.410	51.70731707	
3.90625	0.207	0.410	49.51219512	
1.953125	0.216	0.410	47.31707317	
0.9765625	0.253	0.410	38.29268293	

TABLE 5- IC_{50} VALUES OF *FICUS RACEMOSA* (LEAVES)

Concentration (µg/ml)	Absorbance of extract	Absorbance of blank	% Inhibition	IC50 (µg/ml)
500	0.054	0.410	86.82926829	14.55
250	0.102	0.410	75.12195122	
125	0.155	0.410	62.19512195	
62.5	0.167	0.410	59.26829268	
31.25	0.180	0.410	56.09756098	
15.625	0.201	0.410	50.97560976	
7.8125	0.210	0.410	48.7804878	
3.90625	0.215	0.410	47.56097561	
1.953125	0.230	0.410	43.90243902	
0.9765625	0.240	0.410	41.46341463	

CONCLUSION: The plant has been being used for a long time in herbal medicine without knowing the exact phytopharmacological properties that works against certain disease. *Ficus racemosa* showed good cytotoxicity properties. The methanolic extract of *Ficus racemosa* (seeds) showed antibacterial activity against the gram positive bacteria of *Surcina lutea. Ficus racemosa* (seeds) and *Ficus racemosa* (leaves) shows strong antioxidant properties. The plant can be further screened against various diseases in order to find out its unexplored efficacy and can be a potential source of biologically important drug candidates.

ACKNOWLEDGEMENT: We would like to thank Professor Dr. Ekramul Haque, the Chairman of Pharmacy Department of the "Atish Dipankar University of Science & Technology" to permit us to work in the laboratories of Pharmacy department on the project "Antimicrobial, Cytotoxic and Antioxidant Activity of the seeds and leaves of "*Ficus racemosa*".

During the project, we were helped a lot by Dr. Manirul Islam with his expertise in Phytochemistry. We are also thankful to our friend Md. Farhad Hossain Masum, to help us out in proof reading the manuscript.

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