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In-Vitro Anti-oxidant And Antimicrobial Study Of *Ficus hispida*

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Abstract *Ficus hispida* L. belongs to the Moraceae family and is used by the maaiba trible (indigenous medicine - man of Manipur, India) as an indigenous traditional medicine. Present study deals with the successive extraction of the aerial parts of *Ficus hispida* and in-vitro screening of anti-oxidant and anti-microbial activity. The phytochemical screening of the methanol extract of Ficus hispida shows the presence of secondary metabolite groups like alkaloid, phenolic compounds, flavonoid, glycosides, protein etc. Phenolic compounds are commonly found in both edible and nonedible plants and are responsible for various medicinal activities of plants, so our study is based on determining antioxidant activity and anti-microbial activity. Beside these, we also measured the total flavonoid and total phenolic content of the respective sample to understand the effect of polyphenolic compound on different pathophysiological state associated with high free radical production. The in-vitro investigation proves the efficiency of this plant in various diseases states.

Keywords: *Ficus hispida;* Antioxidant activity; Minimum Inhibitory Concentration; Antimicrobial activity.

1. INTRODUCTION

Worldwide interest in studying traditional systems of medicine and exploring their potency is increasing day by day. Different techniques like morphological, phytochemical, and pharmacological and various chemical screening are employed for evaluation of medicinally active crude drugs. Many medicinally active compounds present in medicinal plants play an

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important role in the prevention of diseases. The free radical scavenging molecules, such as polyphenolic compounds, flavonoids, vitamins, nitrogen compounds, terpenoids and other endogenous metabolites are reported to be rich in antioxidant activity (Zheng *et al.,* 2001). These are proven to be useful in the treatment and prevention of diabetes, cancer, respiratory problems and other disease associated with ageing (Sun *et al.,* 2002).

The genus *Ficus* represents an important group of trees with religious value. The genus *Ficus is having* over 700 species belongs to the family Moraceae. Among the genus *Ficus, Ficus hispida* Linn. is an important plant due to its various pharmacological activities, commonly called as peyatti in Tamil, dumoor in Bengali and gobla in Hindi. Ficus mainly grown in evergreen forest, is of moderate height, also found in moist areas, near banks of many stream, in deciduous forest. It is mainly cultivated for its edible fruits in places like India, Andaman Island, Myanmar, and Srilanka etc. (Ripu *et al.,* 2006).

Different parts of this plant are used tradionally for the treatment of various conditions in India, but from the medicinal point of view leaves are most vital (Nadkarni 1976) as an antidiarrheal, Mandal *et al.,* (2002) hepatoprotective, Mandal *et al.,* (2000) anti-inflammatory, Rastogi *et al.,* (1993) antitussive, antipyretic, astringent, hemostatic and anti-ulcer activity, Nadkarni (1976) and Rastogi *et al.,* (1993).

The fruit is commonly used as a tonic and coolant. The fig juice with jiggery is useful as a mild purgative. A mixture of honey and fig juice is used in the treatment of hemorrhage, Sergio *et al.,* (2002), the root and leaves are have potential antidiarrhoeal activity, Mandal *et al.,* (2002), antidiabetic, Ghosh *et al.,* (2004), anti-bacterial, Kone *et al.,* (2004), and as cardio protective, Shanmugarajan *et al.,* (2008) among others.

Our body defence system is well established against reactive oxygen species (ROS) by the help of antioxidants. The ROS are the harmful by products generated during normal cell aerobic respiration, Salah *et al.,* (1995).

The phenolic compounds have antioxidant activity, due to their redox properties by which they act as hydrogen donors, reducing agents and singlet oxygen quenchers. Phenolics are the secondary plant metabolites that are easily available in the plant kingdom and have abundant applications in cosmetic, food and pharmaceutical industry. Strube *et al.,* (1993) and Kahkonen *et al.,* (1996).

Beside antioxidant activity, phenolic compounds also possess activities like antiallergic, anti-inflammatory, antimicrobial activity, cardioprotective, anti-thrombotic and vasodialattory effect. Balasundram *et al.,* (2006).

The main objectives of the present study was the estimation of antioxidant properties of the methanol extract of leaves of the plant *Ficus hispida* along with evaluation of its anti-microbial property.

2. MATERIALS & METHODS

The plant was collected from Chuchura district on September, 2014, West Bengal & was authenticated (Specimen ID- CNH/Tech.II/2014/81/199) by Botanical Survey of India, Botanic Garden, Shibpur, Howrah.

The sample was shade dried till complete drying & the leaves become brittle. The dried leaves were made into coarse powder by grinder & kept into air tight container.

2.1 Plant profile

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Rosales
Family: Moraceae
Genus: *Ficus*
Species: *Ficus hispida*

Common name: The *Ficus hispida* name is derived from the Latin word FIK-us meaning "for Fig" and HISS-pih- duh meaning"with bristly hairs". Ficus is also known as hairy fig, devil fig, gobla (hindi), dhed umbar(gujrati), kakodambarik(Sanskrit), kothaya-dumari(nepali).

Macroscopic Characters: Leaves are simple, deccusate, opposite, striplesto 2.5*1cm, caducous leaving annular scar; petiole 1-10 cm long, canaliculated, hispid; lamina 7-35*3-16, narrow ovate, elliptic oblong, narrow obovate, apex caudate-acuminate, subcordate or truncate–subcordate, margin entire, scabrid on both surface with hispid beneath. Bark is brownish, lenticellate, blaze pink branchletsterete with hollow internodes, densty hispid with brown or grey hair, lentcellate (Nadkarni, 1976).

2.2 Preparation of plant extract

After collection & identification of the plant *Ficus hispida*, the phytochemical investigations were carried out. Successive extraction of the dried plant was done by different solvents like Petroleum ether (60-80^{0}C), Chloroform & Methanol. Soxhlet apparatus was used for extraction. Each extracts were concentrated by distilling off the solvent. The concentrated extracts were evaporated to complete dryness and were weighed and stored suitably. The percentage yield was calculated in terms of initial air dried basis. The results are tabulated in **Table 1.**

2.3 Phytochemical screenings

The methanolic extract of *Ficus hispida* was subjected to various phyto-chemical test for the identification of chemical constituents and chemical groups present. Kokate *et al.,* and Khandelwal (2012). The results are tabulated in **Table 2.**

2.4 Estimation of Total flavonoid content

Total soluble flavonoid content of the methanol fraction of Ficus was determined using aluminium nitrate and Quercetin as the standard (Hsu *et al.,* 2006). Fraction was prepared by adding 1mg of extract to 1ml of 80 % ethanol. 0.5 ml of the prepared fraction was added to test tubes containing 0.1 ml of 10 % aluminium nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80 % ethanol. The absorbance of the solution was measured at 415 nm after incubating at room temperature for 40 min. The total flavonoid content was expressed as µg/ml of Quercetin equivalent by using the standard quercetin graph based on the calibration curve: $A = 0.0067C + 0.0132$, $R^2 = 0.999$, where A is the absorbance, and C is Quercetin equivalents (µg). The test was performed in triplicate and average value was represented. The results are tabulated in **Table 3.**

2.5 Estimation of Total phenolic content

The total concentration of phenolics in methanol extract of leaves of *Ficus hispida* was determined according to the method (Singleton *et al.,* 1999). Briefly, 0.1 ml of each extract solution (contains 500 µg of extract) was transferred to a 100 ml volumetric flask, and then the finally adjusted with 46 ml of distilled water. Afterward, 1 ml of Folin-Ciocalteu reagent was added into this mixture and after 3 min 3 ml of sodium carbonate (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature, and then absorbance was measured at 760 nm. Pyrocatechol (Sigma) was used as the standard for the calibration curve. The estimation of phenolics in the fractions was carried out in triplicate, and the results were averaged. The phenolic compound content was determined as pyrocatechol equivalents using the standard equation based on the calibration curve: $A = 0.0034C - 0.058$, $R^2 = 0.9996$. A is the absorbance, and C is pyrocatechol equivalents (µg). The test was performed in triplicate and average value was represented. The results are tabulated in **Table 3.**

3. ANTIOXIDANT SCREENING

3.1 DPPH radical scavenging activity

The DPPH (1,1-diphenyl-1-2-picryl hydrazil) free radical scavenging activity of Methanol extract of *Ficus hispida* was measured by employing the method

(Blois, 1958). 1ml of 0.1 mM methanol solution of DPPH was added to 3 ml of various concentrations of drug extract such as (50, 100, 150, 200, 250, 300 µg/ml). Absorbance was measured at 517 nm after 30min. The percentage of inhibition was calculated by comparing the absorbance values of the control & test samples. All the tests were performed in triplicate.

The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{Percentage DPPH scavenging activity} = (A_{control} - A_{test})/A_{control}$$

Where $A_{control}$ is the absorbance of the control & A_{test} is the absorbance in the presence of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of drug extract that inhibits the formation of DPPH radicals by 50%. The results are tabulated in **Table 4**

3.2 Hydrogen Peroxide (H_2O_2) radical scavenging activity

The ability of methanol extract of *Ficus hispida* to scavenge H_2O_2 was determined according to the method of Ruch *et al.,* (1989). A 40 mM phosphate buffer solution of H_2O_2 was prepared in (pH 7.4). The drug extract of the following concentrations (50, 100, 150, 200, 250, 300 µg/ml) in distilled water were added to a 0.6 ml, 40 mM H_2O_2 solution. After 10min later at 230 nm absorbance was taken, against a blank solution containing the phosphate buffer without H_2O_2. Ascorbic acid was used as a standard anti-oxidant. The percentage of H_2O_2 scavenging of methanol drug extracts & standard compounds was calculated. Scavenging of both the extracts & standard compounds was calculated. The percentage inhibition was calculated as:

$$\% \ H_2O_2 \text{ radical scavenging activity} = (A_{control} - A_{test})/A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction &A_{test} is the absorbance of the sample of the extracts. The antioxidant activity of the extracts was expressed as IC_{50}. The results are tabulated in **Table 5**

3.3 Nitric Oxide radical ($N0^-$) scavenging activity

Nitric oxide generated from sodium nitroprusside was measured by Greiss reaction where sodium nitroprusside in aqueous solution at physiological pH generates nitric oxide, Green *et al.,* 1982 and Marcocci *et al.,* 1994a, b) which interacts with oxygen to produce nitrite ions that can be estimated by using Greiss reagent. Scavengers of nitric oxide radical involves in the competition with the oxygen leading to reduced production of nitric oxide, Marcocci *et al.,*

(1994a, b). Sodium nitroprusside (5mM) in phosphate-buffered saline (PBS) was mixed with 3.0 ml of different drug extract of the following concentrations (50, 100, 150, 200, 250, 300 µg/) & incubated at 25°C for 2hr 30 min. The drug extracts reacted with Greiss reagent (1% sulphanilamide, 2% H_3PO_4 & 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide following coupling with napthylethylenediamine was determined at 546 nm & compared to the absorbance of standard solutions of potassium nitrite with Greiss reagent. Ascorbic acid was used as a reference compound. The percentage inhibition was calculated as:

$$\% \text{ NO scavenging activity} = (A_{control} - A_{test})/A_{control}$$

Where $A_{control}$ is the absorbance of the control reaction &A_{test} is the absorbance of the sample drug extracts. The antioxidant activity of the extracts was expressed as IC_{50}. The results are tabulated in **Table 6**

4. ANTI-MICROBIAL SCREENING

Materials required: Methanol extract of Ficus, BOD incubator, hot air oven, laminar air flow system, pH paper.

Chemicals required: Muller Hinton Broth (MHB), Muller Hinton Agar (MHA), Isosaline (0.9% w/v Nacl water), and 0.1 (N) NaOH, McFarland standard solution.

Test organisms used:

Gram(+ve) Bacteria	Gram(-ve) Bacteria
1. *Salmonella typhi* NCTC-74	6. *Staphylococcus aureus* ML-357
2. *Salmonella typhi* B-111	7. *Staphylococcus aureus* ML-15
3. *Salmonella typhi* C-145	8. *Staphylococcus aureus* ML-366
4. *Salmonella typhi* E-3404	9. *Staphylococcus aureus* ML-276
5. *Salmonella typhi* A-2467	10. *Staphylococcus aureus* ML-145

4.1 Procedure

Preparation of bacteria strains above mentioned-

i) All the strains were sub cultured in freshly prepared MHB in an incubator for 24 hours at 37^{0}C.

ii) The cultured bacterial strains were diluted with normal saline with reference to McFarland Standard.

4.2 Preparation of drug plate for MIC (Minimum inhibitory concentration) test

i) Different concentrations of the methanolic extract of Ficus were prepared as following 1mg/ml, 2.5mg/ml, 5mg/ml, 10mg/ml and 25mg/ml.
ii) The weighed drug extracts were dissolved in sterile water.
iii) The different concentration were then mixed with freshly prepared sterile Muller Hinton Agar & dispersed in 45 mm petridish.
iv) The back of the petridishes were marked from 1-10.
v) One petridish was left blank without the drug extract.

4.3 Inoculation

i) The petridishes were inoculated with the test organisms.
ii) Then the petridishes were left overnight in a BOD incubator at 37^0C.
iv) The next day results were obtained. The results are tabulated in **Table 7 and Table 8**

5. RESULTS AND DISCUSSION

The yield value shows that the methanol extract shows maximum yield value.

Table 1: Yield values of extracts of *Ficus hispida.*

Solvent system	Weight of pet. disc(gm)	Weight of pet. disc+drug(gm)	Weight of drug (gm)
Pet ether	38.92	40.37	1.25
Choloroform	44.62	45.15	0.53
Methanol	46.31	47.76	1.35

The phytochemical analysis shows the presence of phenolic compound, glycoside, flavonoid etc.

Table 2: Phytochemical screenings of methanol extract of *Ficus hispida.*

Groups	Present/Absent
Alkaloids	absent
Flavonoids	present
Tannin	Present
Steroids	present
Amino acid	absent
Glycoside	present
Saponin	present

Table 3: Calculation of total phenolic content and total flavonoid content of methanol extract of *Ficus hispida.*

Methanol extract of Ficus hispida	Total flavonoid content (mg/gm equivalent)	Total phenolic content (mg/gm equivalent)
	27.134	47.64

6. IN-VITRO ANTI-OXIDANT ASSAY

Table 4: DPPH free radical scavenging activity of methanol extract of *Ficus hispida.*

Concentration of drug(µg/ml)	%Scavenging activity	IC $_{50}$ Value(µg/ml)
0	0	
50	40.67	160.80
100	55.25	
150	73.22	
200	55.59	
250	43.38	
300	70.16	

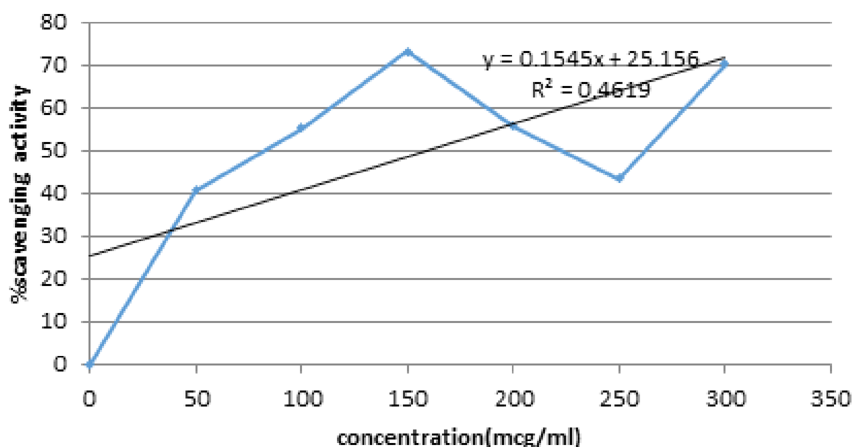

Figure 2: DPPH radical scavenging activity of methanol extract of *Ficus hispida.*

Table 5: Hydrogen Peroxide (H_2O_2) free radical scavenging activity of methanol extract of *Ficus hispida.*

Concentration of drug(µg/ml)	%Scavenging activity	IC $_{50}$ Value(µg/ml)
0	0	
50	24.86	218.75
100	28.91	
150	35.35	
200	41.25	
250	59.30	
300	65.19	

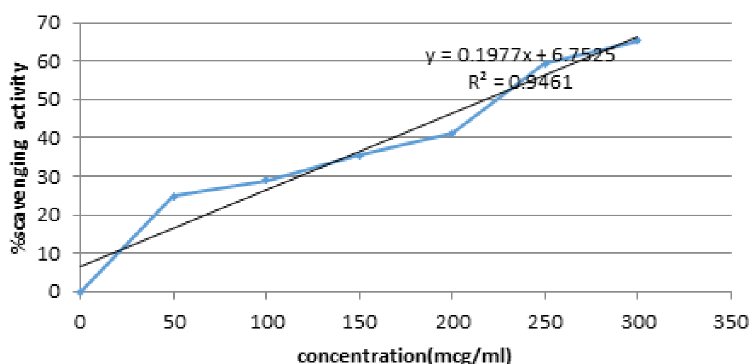

Figure 3: Hydrogen Peroxide (H_2O_2) radical scavenging activity of methanol extract of *Ficus hispida.*

Table 6: Nitric Oxide radical (NO^-) free radical scavenging activity of methanol extract of *Ficus hispida.*

Concentration of drug(µg/ml)	%Scavenging activity	IC_{50} Value(µg/ml)
0	0	
50	48.06	350.83
100	59.66	
150	62.43	
200	80.11	
250	90.60	
300	98.34	

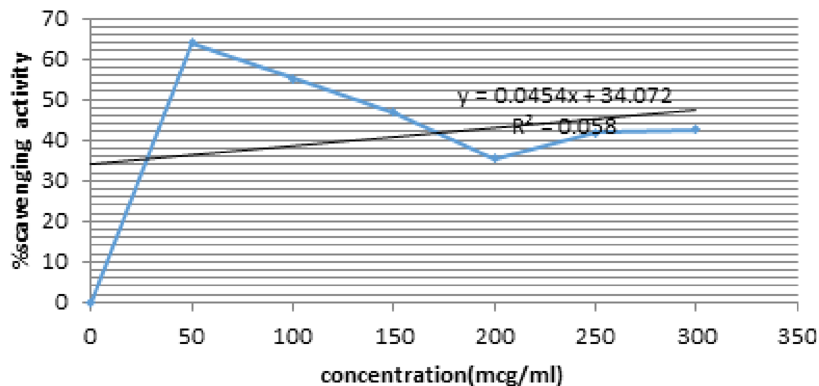

Figure 4: Nitric Oxide radical (NO^-) scavenging activity of methanol extract of *Ficus hispid.*

6.1 Antimicrobial screening

Table 7: Shows the presence and absence of different gram +ve and gram –ve bacteria in methanol extract of *Ficus hispida.*

Strain no.	Drug Concentration					
	Blank	1mg/ml	2.5mg/ml	5mg/ml	10mg/ml	25mg/ml
1	+	+	+	+	-	-
2	+	+	+	+	+	+
3	+	+	+	+	-	-
4	+	+	+	-	-	-
5	+	-	-	-	-	-
6	+	+	+	+	+	-
7	+	+	+	+	-	-
8	+	+	+	+	+	-
9	+	+	+	+	+	-
10	+	+	+	+	+	+

(+)Sign indicate positive growth

(-)Sign indicate negative growth

Table 8: The table shows MIC (Minimum Inhibitory Concentration) of FH against bacteria strains

Strain no.	Bacteria strain	MIC (mg/ml)
1	Salmonella typhi NCTC-74	10
2	Salmonella typhi B-111	-
3	Salmonella typhi C-145	10
4	Salmonella typhi E-3404	05
5	Salmonella typhi A-2467	01
6	*Staphylococcus aureus* ML-357	25
7	*Staphylococcus aureus* ML-15	10
8	*Staphylococcus aureus* ML-366	25
9	*Staphylococcus aureus* ML-276	25
10	*Staphylococcus aureus* ML-145	-

CONCLUSION

Production of free radical in the body is responsible for various pathological conditions. The conversion of oxygen to reactive oxygen species (ROS) may promote uncontrolled reactions. The reason behind this is mainly due to exposure to radiation, chemicals or by other means. Antioxidants play an important role in building resistance against this reactive oxygen species (ROS) by scavenging free radical, inhibiting lipid peroxidation etc.

Polyphenols are antioxidants with redox properties, which permit them to perform as reducing agents, hydrogen donators, and singlet oxygen quenchers. Some show metal chelation properties. In addition, some have antimicrobial activity, Hatano *et al.,* (1985) and Tsao, *et al.,* (2005). Thus they may contribute to the antioxidant action and have inhibitory effects on mutagenesis and carcinogenesis in human beings, Tsao, *et al.,* (2005). The preliminary phytochemical screening shows the presence of alkaloid, saponins, glycosides, flavonoid, phenols etc. Yoshiki *et al.,* (1995), Yoshiki *et al.,* (1998) and Hu *et al.,* (2005), in different plant extracts. Methanol extract of *Ficus hispida* is found to contain substantial amount of phenolic compound and flavonoid. It is found that the TPC (Total Phenolic Content) is 47.64 mg of Catechol equivalent per gm of extract and TFC (Total Flavonoid Content) is 27.13 mg of Quercetin equivalent per gm of extract, which may contribute to the antioxidant and antimicrobial activity.

The methanol extract of *Ficus hispida* produces substantial amount of antioxidant activity. The plant extracts exhibit *in-vitro* free radical scavenging activity against DPPH, H_2O_2 and Nitric oxide, having numerous physiological effects and also used in various pathological conditions. The IC_{50} value of DPPH, H_2O_2, and Nitric Oxide radical (NO^-) radical scavenging activity of methanol extract of *Ficus hispida* is 160.80µg/ml,218.75µg/ml and 350.83µg/ml respectively. Those observations indicate that the methanol extract of *Ficus hispida* have significant antioxidant activity.

The methanol extracts of *Ficus hispida* possess inhibitory effects against various Gram positive and Gram negative organisms. It also possesses considerable anti-inflammatory activity as shown in the data depicted in the results section.

The methanol extract of *Ficus hispida* is reported to be rich in saponins, Singh *et al.,* (1997). The presence of saponins in the methanol extract is confirmed by the preliminary phytochemical screening. Saponins have been shown to possess antioxidant property, Yoshiki *et al.,* (1995), Yoshiki *et al.,* (1998) and Hu *et al.,* (2005). Further studies have confirmed that oxidative stress plays an important role in the initiation and progression of liver disease, Davlo *et al.,* (1998) and Arteel (2009). *Ficus hispida* contains considerable amount of Saponins, so it can be correlated with its hepatoprotective activity.

From the above observations it is well reflect that the methanol extract of *Ficus hispida* is having prompt antioxidant and antimicrobial property. We already know various hepatic enzymes are effective against those free radicals. We suggest that natural antioxidant and scavenging agents in *Ficus hispida* extract might be effective as plant hepatprotectors and interference of those free radical scavengers on hepatic enzymes can be studied in details. Along with this the plant extract can also be tested for anti-inflammatory property as it is having potent antioxidant activity.

Further studies regarding isolation and characterization of active principles responsible for antioxidant, antimicrobial activity is currently under progress.

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