IN VITRO FREE RADICAL SCAVENGING ACTIVITY OF *FICUS BENGHALENSIS* LINN. AND *FICUS RACEMOSA* LINN. LEAF EXTRACTS

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Summary

Antioxidant activity of alcoholic and aqueous extracts of *Ficus benghalensis* leaf (FBL) (Moraceae) and *Ficus racemosa* (Moraceae) leaf (FRL) extracts were carried out by Hydrogen peroxide (H_2O_2), Diphenyl Picryl Hydrazyl (DPPH) and nitric oxide scavenging activity. Ascorbic acid was taken as standard. Alcoholic extract have significant free radical scavenging activity. In Hydrogen sulfide scavenging assay, IC_{50} value of alcoholic extract of FBL, alcoholic extract of FRL and ascorbic acid is 399.5µg/ml, 461µg/ml and 276µg/ml respectively. In Diphenyl Picryl Hydrazyl scavenging assay, IC_{50} value of alcoholic extracts of FBL, FRL and ascorbic acid is 158.2µg/ml, 122 µg/ml and 104.2µg/ml respectively. In Nitric oxide scavenging assay, IC_{50} value of alcoholic extracts FBL, FRL and ascorbic acid is 373µg/ml, 343.4 µg/ml and 264.5µg/ml respectively. Flavonoid content of FBL alcoholic extract was 0.18% and FRL alcoholic extract was 0.14% of catechin equivalent of 100 g of fresh mass. Phenolic content in FBL alcoholic and aqueous extract was 0.45% and 0.09% of gallic acid equivalents of 100 g fresh mass respectively. Phenolic content in FRL alcoholic and aqueous extract was 0.37% and 0.04% of gallic acid equivalents of 100 g fresh mass respectively. The reducing power is also comparable to ascorbic acid and this activity may be because of flavonoids and phenolic compounds present in alcoholic extract.

Key words: antioxidant, hydrogen peroxide, nitric oxide, Diphenyl Picryl Hydrazyl, *Ficus benghalensis, Ficus racemosa*

Introduction

Ficus racemosa is also known as “Gular”. Powder leaves mixed with honey are given in vitiated conditions of pitta. A decoction of the leaves is a good wash for wounds and ulcers (1). Ficus racemosa root also have in vitro free radical scavenging activity (2). It also shows antilipid peroxidation effects. (3) Leaves of ficus racemosa also have hepatoprotective effect **(**4). Ficus benghalensis is also known as “Bannayan tree”. This plant has hypoglycemic activity (5). According to Unani system of medicine, latex is maturant, lessens inflammations, aphrodisiac, tonic, vulernary and is useful in piles.

According to Ayurveda, it is astringent to bowels; useful in treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy. (6). Ficus benghalensis also have potential wound healing activity (7). Based on above mentioned studies and ethanobotanical uses, the free radical activity of *Ficus benghalensis and Ficus racemosa* leaf was carried out.

Materials and Methods

Plant material

Ficus benghalensis Linn. (FBL) and *Ficus racemosa* Linn.leaves (FRL) were procured from south gujarat region, with the help of local tribal and field botanist. Care was taken to select healthy plant for normal leaves. The plant material was identified by department of Bioscience, VNSGU, Gujarat (voucher specimen no. MAP/08/08, MAP/09/08). The leaves were dried under the shade. The dried leaves were powdered and were passed through the sieve no. 60.

Preparation of Extract

Preparation of the extract of powdered leaves is done using alcohol and distilled water. The shade dried coarse powder of the leaves (500 gm) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 90% alcohol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely. It is dried and kept in a desiccator till experimentation. Similarly, aqueous extract was prepared.

Qualitative phytochemical analysis

Alcoholic and aqueous extracts of FBL, FBL both the leaves were subjected to various qualitative tests for the identification of phytoconstituents. (8).

Determination of Total flavonoids

Flavonoid content was determined only in alcoholic extract. Aluminum chloride colorimetric method was used for flavonoids determination. 1 ml of sample was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at various concentrations in methanol. The total flavonoid content was expressed as percentage of catechin equivalent per 100 g fresh mass. (9).

Determination of total Phenolic content

Total soluble phenolics in the aqueous and alcoholic extracts FRL were determined with Folin–Ciocalteu reagent, according to the method pyrocatechol taken as a standard. 1 ml of extract solution in a volumetric flask was diluted glass-distilled water (46 ml). Folin–Ciocalteu reagent (1 ml) was added and the contents of the flask were mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added, then the mixture was allowed to stand for 2 hr with intermittent shaking. The absorbance was measured at 760 nm. Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g fresh mass. (10).

Hydrogen Peroxide radical scavenging activity

1 ml of extract solution [(100 to 600µg/ml prepared in phosphate buffer saline (PBS)] was incubated with 0.6 ml of 40mM H_2O_2 solution (prepared in PBS) for 10 minutes. The absorbance of the solution was carried at 230nm. H_2O_2 alone was taken as blank. Ascorbic acid was taken as standard (10). The radical scavenging activity was calculated as

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

Diphenyl Picryl Hydrazyl (DPPH) radical scavenging activity

One thousand microlitres of diverse concentrations (40 to 200µg/ml) of extracts were added to 4 ml of 0.004% methanol solution of DPPH (1, 1 -diphenyl-2-picrylhydrazyl). After 30 minutes of incubation period at room temperature, the absorbance was read against a blank at 517nm (10). Ascorbic acid was taken as the standard. Inhibition of free radical by DPPH in percent was calculated in following way

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

Nitric Oxide radical scavenging activity

2 ml of Sodium nitroprusside (10mM) in phosphate buffer saline (pH 7.4) was diversed with different concentrations of extract (100 to 600µg/ml), dissolved in suitable solvent and incubated at room temperature for 150 minutes. The same reaction mixture without extract served as control. After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N - (1-naphthyl) ethylene diamine dihydrochloride) was added. The absorbance was measured at 546nm (10). Ascorbic acid was taken as standard. The nitric oxide radical scavenging activity was calculated as

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

Determination of reducing power

Fe^+ reducing power was determined only with alcoholic extract. The extract (0.75 ml) at various concentrations was mixed with 0.75 ml of phosphate buffer (0.2 M, pH 6.6) and 0.75 ml of potassium hexacyanoferrate ($K_3Fe(CN)_6$) (1%, w/v), followed by incubating at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.75 ml of trichloroacetic acid (TCA) solution (10%) and then centrifuged at 800g for 10 min. 1.5 ml of the supernatant was mixed with 1.5 ml of distilled water and 0.1 ml of ferric chloride ($FeCl_3$) solution (0.1%, w/v) for 10 min. The absorbance at 700 nm was measured as the reducing power. Higher absorbance of the reaction mixture indicated greater reducing power (10).

Results

Yield of aqueous extract of FBL was 8.2% and alcoholic extract of FBL was 10.3%. Yield of aqueous extract of FRL was 7% and alcoholic extract of FBL was 10%. Flavonoid content of FBL alcoholic extract was 0.18% and FRL alcoholic extract was 0.14% of catechin equivalent of 100 g of fresh mass. Phenolic content in FBL alcoholic and aqueous extract was 0.45% and 0.09% of gallic acid equivalents of 100 g fresh mass respectively. Phenolic content in FRL alcoholic and aqueous extract was 0.37% and 0.04% of gallic acid equivalents of 100 g fresh mass respectively.

Table 1: Phytochemical screening of aqueous and alcoholic extract of *Ficus Religiosa* Linn. leaf

Plant constituents	Aqueous extract of FBL	Alcoholic extract of FBL	Aqueous extract of FRL	Alcoholic extract of FRL
Alkaloids	-	-	-	-
Saponins	-	-	-	-
Glycosides	+	-	-	-
Carbohydrates	+	+	+	+
Tannins and Phenolic compounds	+	+	+	+
Flavonoids	+	+	+	+
Steroids	+	+	+	+
Proteins and Amino acids	-	-	–	–
Triterpenoids	+	+	_	_

Table 2: hydrogen peroxide radical scavenging activity of aqueous and alcoholic extract of *Ficus benghalensis* Linn. leaf

Sr. No.	Concentration (µg/ml)	% Inhibition		
		aqueous extract	alcoholic extract	ascorbic acid
0	0	0	0	0
1	100	7.3 ± 0.1	15.9 ± 0.9	35.1 ± 0.8
2	200	9 ± 0.9	27.5 ± 1.3	49 ± 2.9
3	300	12.3 ± 1.3	35.2 ± 2	58 ± 3.1
4	400	14.1 ± 2.1	53.3 ± 2.3	68.5 ± 2.9
5	500	16 ± 2.4	65.9 ± 3.1	77.9 ± 2.5
6	600	18.8 ± 2.6	69.1 ± 4.2	84.1 ± 3.5

Values are the average of six experiments and represented as mean ± SEM

Table 3: DPPH radical scavenging activity of aqueous and alcoholic extract of *Ficus benghalensis* Linn. leaf

Sr. No.	Concentration (µg/ml)	% Inhibition		
		aqueous extract	alcoholic extract	ascorbic acid
1	0	0	0	0
2	40	5.5 ± 0.1	14.8 ± 0.9	26.8 ± 0.8
3	80	8.1 ± 0.9	23.1 ± 1.3	40.3 ± 2.9
4	120	11.1 ± 1.3	31.2 ± 2	57.6 ± 3.1
5	160	14.2 ± 2.1	50.6 ± 2.3	75.2 ± 2.9
6	200	18.5 ± 2.4	64.3 ± 3.1	89.1 ± 2.5

Values are the average of six experiments and represented as mean ± SEM

Table 4: nitric oxide scavenging activity of aqueous and alcoholic extract of *Ficus benghalensis* Linn. leaf

Sr. No.	Concentration (µg/ml)	% Inhibition		
		aqueous extract	alcoholic extract	ascorbic acid
1	0	0	0	0

2	100	6.3 ± 0.1	20.6 ± 0.9	35.1 ± 0.8
3	200	8.9 ± 0.9	39.1 ± 1.3	48.6 ± 2.9
4	300	12.6 ± 1.3	41.5 ± 2	56.2 ± 3.1
5	400	18.2 ± 2.1	55.3 ± 2.3	69.4 ± 2.9
6	500	25.1 ± 2.4	64.6 ± 3.1	78.2 ± 2.5
7	600	29.6 ± 2.6	70.5 ± 4.2	86.1 ± 3.5

Values are the average of six experiments and represented as mean ± SEM

Table 5: hydrogen peroxide radical scavenging activity of aqueous and alcoholic extract **of *Ficus racemosa* Linn. leaf**

Sr. No.	Concentration (µg/ml)	% Inhibition		
		aqueous extract	alcoholic extract	ascorbic acid
0	0	0	0	0
1	100	6.1 ± 0.1	13.9 ± 0.9	35.1 ± 0.8
2	200	7.3 ± 0.9	28.3 ± 1.3	49 ± 2.9
3	300	9.6 ± 1.3	32.1± 2	58.1 ± 3.1
4	400	12.6 ± 2.1	47.1 ± 2.3	68.5 ± 2.9
5	500	14.1 ± 2.4	52.1 ±3.1	77.9 ± 2.5
6	600	15 ± 2.6	62.3 ± 4.2	84.1 ± 2.5

Values are the average of six experiments and represented as mean ± SEM

Table 6: DPPH radical scavenging activity of aqueous and alcoholic extract of *Ficus racemosa* Linn. leaf

Sr. No.	Concentration (µg/ml)	% Inhibition		
		aqueous extract	alcoholic extract	ascorbic acid
1	0	0	0	0
2	40	6.9 ± 0.1	23.9 ± 0.9	26.8 ± 0.8
3	80	7.8 ± 0.9	31.5 ±1.3	40.3 ± 2.9
4	120	12.1 ±1.3	51.9 ± 2	57.6 ± 3.1
5	160	14.5 ± 2.1	63.4 ±2.3	75.2 ± 2.9
6	200	17.3 ± 2.4	78 ± 3.1	89.1 ± 2.5

Values are the average of six experiments and represented as mean ± SEM

Table 7: nitric oxide scavenging activity of aqueous and alcoholic extract of *Ficus racemosa* Linn. leaf

Sr. No.	Concentration (µg/ml)	% Inhibition		
		aqueous extract	alcoholic extract	ascorbic acid
1	0	0	0	0
2	100	5.1 ± 0.1	22 ± 0.9	35.1 ± 0.8
3	200	7.3 ± 0.9	38.2 ±1.3	48.6 ± 2.9
4	300	13.8 ± 1.3	47.3 ± 2	56.2 ± 3.1
5	400	16.7 ± 2.1	57.4 ± 2.3	69.4 ± 2.9
6	500	21.3 ± 2.4	64.8 ±3.1	78.2 ± 2.5
7	600	25.9 ± 2.6	73.1 ± 4.2	86.1 ± 3.5

Values are the average of six experiments and represented as mean ± SEM

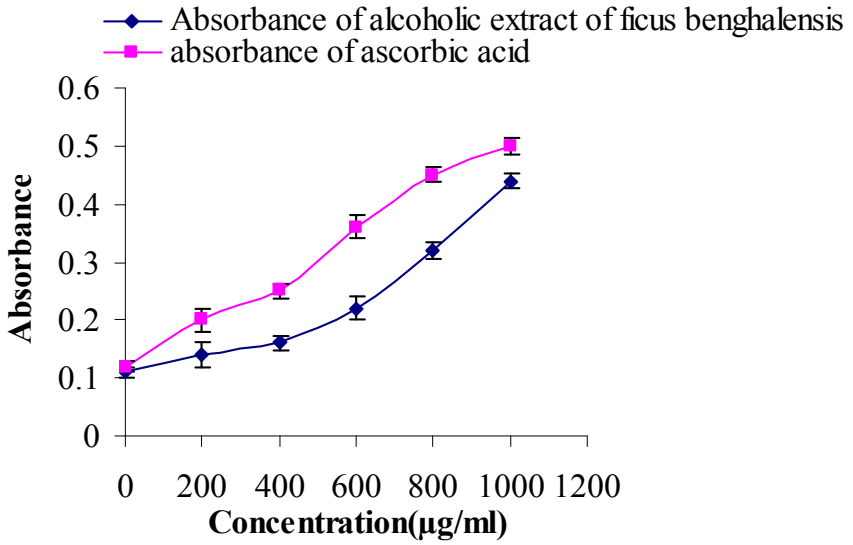

Figure 1: Reducing power of *Ficus benghalensis* Linn. leaf compared to ascorbic acid.
Values are the average of triplicate experiments and represented as mean ± standard deviation

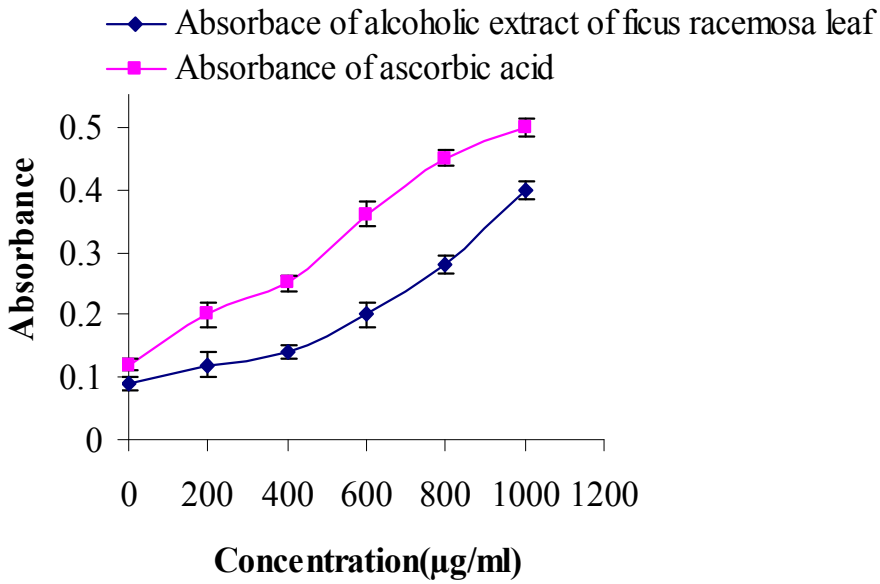

Figure 2: Reducing power of *Ficus racemosa* Linn. leaf compared to ascorbic acid.
Values are the average of triplicate experiments and represented as mean ± standard deviation

Discussion

In H_2O_2 scavenging assay, aqueous extract of both the plant did not showed significant scavenging activity. Alcoholic extracts and Ascorbic acid exhibited significant hydrogen peroxide scavenging activity. IC_{50} value of alcoholic extract of FBL, alcoholic extract of FRL and ascorbic acid is 399.5µg/ml, 461µg/ml and 276µg/ml respectively (Table 2, 5).

Also in DPPH scavenging assay, aqueous extract of both plants did not showed significant scavenging activity. IC_{50} value of alcoholic extracts of FBL, FRL and ascorbic acid is 158.2µg/ml, 122 µg/ml and 104.2µg/ml respectively (Table 3, 6). In Nitric oxide scavenging assay, aqueous extract of both the plants did not showed significant scavenging activity. IC_{50} value of alcoholic extracts FBL, FRL and ascorbic acid is 373µg/ml, 343.4 µg/ml and 264.5µg/ml respectively (Table 4, 7). These results showed that the alcoholic extract of FBL and FRL has significant free radical scavenging activity. This activity is may be due to flavonoids and phenolic compounds present in alcoholic extract (11). Aqueous extract also Phenolic compounds but did not showed significant activity may be because of very low content. The presence of antioxidant in alcoholic extract causes the reduction of the Fe^{+}/ ferricyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 1 and 2 shows the reductive capabilities of the plant extract compared to ascorbic acid. The reducing power of extract of FBL and FRL was very potent and the power of the extract was increased with quantity of sample. Leaves of both the plants are widely used in wound healing. Wound healing property of this plant is may be because of inhibition of lipid peroxidation. Inhibition of lipid peroxidation stimulates wound healing (12). Inhibition of lipid peroxidation is may be because of antioxidant activity of this plant. This plant also has anti-inflammatory activity that may be also because of free radical scavenging activity of flavonoids and anti-inflammatory property of flavonoids and terpenoids (13, 14). This study proves antioxidant acitivy of *Ficus benghalensis* and *Ficus racemosa.* Linn leaves, which may responsible mechanism behind its ethanobotanical uses.

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