In-Vitro Antioxidant Activity of Methanolic Extraction of *Ficus Benghalensis L.* Latex

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Summary

Reacting active species induced oxidative damage of cellular tissue cause to many human diseases like cancer, cardiovascular disease, nephropathy and aging. Naturally occurring antioxidant supplements from plants are vital to counter the oxidative damage in cells. The main objective of the present study was to investigate the in-vivo antioxidant potential methanolic extraction of *Ficus benghalensis L* latex. The extract was used study their phytochemical composition, total Phenolic content , flavonoid contents, and in vitro antioxidant activities including 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, ferric chloride scavenging, and phosphor-molybdenum scavenging activity. Finally percentage of inhibition of free radical and IC_{50} were calculated the by the help Statistical analysis. The phytochemical studies of the methanolic extraction of *Ficus benghalensis L* latex have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids, amino acids and total Phenolic content (276±0.84mg GAE/gm extract) and total flavonoid content (1.84±0.5 mg QE/gm). The IC_{50} values for scavenging DPPH, ferric chloride, phosphor-molybdenum were 28.63±0.16 µg/ml, 49.82 ±1.00µg/ml and 31.84±0.12 µg/ml respectively. The results of present data was shown that the methanolic extraction of *Ficus benghalensis L* latex have contributed high potential in-vitro antioxidant activity.

Keywords: Gallic acid: Quercetin: DPPH: TPTZ: Trolox.

Introduction

Free radical and Reactive oxygen species (ROS) like superoxide, hydroxyl radical peroxyl radical as well as non-radical species such as hydrogen peroxide (H2O2) [1]. These free are derived from the normal metabolism or exogenous agent like chemical or medicine (2). Reactive species are act as oxidative damage of cellular tissue that is implicated as a possible factor in the etiology of several human diseases, including cancer, cardiovascular disease, and aging (3). *In vivo*; such reactive species is reduced by endogenous antioxidative defences, so as to preserve optimal cellular function. In pathological conditions, however, the detoxifying mechanisms are often inadequate as excessive in finding antioxidant phytochemical, because they can inhibit the propagation of free radical reactions; protect the human body from diseases [4]. The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, and singlet oxygen quenchers and metal chelators [5]. The preset study was evaluated in - vitro antioxidant potential of methanolic extraction of *Ficus benghalensis L* latex. Because *Ficus benghalensis* **(**family-Moraceae) contain glycoside, 20-tetratriaconthene-2-one, 6-heptatriacontene-10-one,

pentatriacontan-5-one, beta sitosterol-alpha-D-glucose, and meso-inositol have been isolated from the bark of the *Ficus benghalensis* (6). The fruit extracts exhibited antitumor activity in the potato disc bioassay (7). The leaves contain 9.63% crude protein, 26.84% crude fibres, 2.53% CaO, and 0.4% Phosphorous. It yields latex containing Caoytchoue (2.4%), Resin, Albumin, Cerin, sugar, and Malic acid. It is used in ayurveda for the treatment of diarrhea, dysentery, and piles (8) and as a hypoglycemic. (9&10). So it may have antioxidant potential. Thus, the purpose of current study was investigate the in - vitro antioxidant potential of methanolic extraction of *Ficus benghalensis L* latex.

Materials and methods

Drug and Reagents

Folin-Ciocalteus's phenol reagent, sodium carbonate, Gallic acid (GA), Quercetin (QE), FeCl3, NaNO2, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid DPPH· (1,1-Diphenyl-2- TPTZ (2,4,6-Tripyridyl-s-triazine), Trolox, BHT (Butylated hydroxytoluene) were purchased from Sigma Chemical Company Ltd, and Sodium nitro preside (SNP), a-napthyl-ethylenediamine, potassium ferricyanide, trichloroacetic acid were purchased from Merck pvt. Ltd., India). All the chemicals used including the solvents, were of analytical grade.

Plant material

Ficus benghalensis L latex was collected from village pipariya, dist. Vadodara (G.P., India). The plant was identified by Dr. Nagar (Professor of botany), M.S. University vadodara (Gujarat) and voucher specimen (DPSV/F/01/2010) was submitted in department of Pharmacy, Sumandeep Vidyapeeth Vadodara, Gujarat.

Sample Preparation and Extraction

The *Ficus benghalensis* L latex was extracted using methyl alcohol as a solvent. The extract was dried by rotator evaporator under reduced pressure.

Photochemical Screening

Standard phytochemical methods were used to test for the presence of saponins, alkaloids, tannins, anthraquinones, cardiac glycosides, cyanogenetic glycosides, amino acid & protein and flavonoids (11, 12, 13, 14, 15, and 16) (Shown table no. 1)

Determination of total phenolic content

The 100 mg pure Gallic acid was dissolved in 100 ml doubled distilled water then it was further dilution in µl to made five different concentration solutions such as 10µl, 20µl, 30 µl, 40µl, 50µl respectively. Then absorbance was taken for respective concentration of standard solution at 629 nm wavelength by U.V. spectrophotometer, and then standard curve was plotted with help of various concentration and absorbance. The extract was dissolved in doubled distilled water and was made up 100µl dilution and was added respective ingredient in above each step of procedure. Further absorbance was taken same as per standard at 629nm (Taga et al., 1984) (17).

Determination of total Flavonoids content

The 100 mg pure quercetin was dissolved in 100 ml doubled distilled water then further dilution in µl and was made five different concentration solutions such as 10µl, 20µl, 30 µl, 40µl, 50µl respectively. Then absorbance was taken of respective concentration of standard solution at 419 nm wavelength by U.V. spectrophotometer, then standard curve was plotted with help various concentration and absorbance. The 100 mg pure quercetin was dissolved in 100 ml doubled distilled water then further dilution in µl and was made five different concentration solutions such as 10µl, 20µl, 30 µl, 40µl, 50µl respectively. Then absorbance was taken of respective concentration of standard solution at 419 nm wavelength by U.V.

spectrophotometer, then standard curve was plotted with help various concentration and absorbance (Jia**,** et al.**,** 1999) (18)

Antioxidant activity:

Determination of DPPH scavenging *assay:*
DPPH radical scavenging activity of *Ficus benghalensis* L latex was determine according to the method reported by Blois [19]. An aliquot of 0.5 ml of sample solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 min in the dark at room temperature. The absorbance was measured at 517 nm using UV spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using the formula.
% of inhibition = absorbance of control – absorbance of sample / absorbance of control ×100

Determination of Fecl3 scavenging Antioxidant assay (FSAA):
The ferric chloride scavenging assay was performed according to Benzie and Strain (20) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g CH3COONa.3H2O and 16 ml CH3COOH), pH 3.6, 10 mM TPTZ (2, 4, 6-Tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM FeCl3.6H2O solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml FeCl3.6H2O solution and then warmed at 37°C before using. The solutions of plant samples and trolox were formed in methanol (250 mg/mL). 10 mL of each of sample solution and BHT solution were taken in separate test tubes and 2990 mL of FSAA solution was added in each to make total volume up to 3 mL. The plant samples were allowed to react with FSAA solution in the dark for 30 min. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 595 nm. The FRAA values were determined as micromoles of trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of trolox. Results were expressed in TE µg/mL.

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Determination of phosphor- molybdenum scavenging assay:
The antioxidant activity of the methanolic extract was determined by the phosphor-molybdenum Method as described by Prieto *et al*, [21]. 0.3 ml of extract was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mMammonium molybdate). The reaction mixture was incubated at 95ċ for 90 min and cooled to room temperature. Finally, absorbance was measured at 695 nm using a spectrophotometer (Merck thermo spectronic, Model NO. UV-1, double beam) against blank. Methanol (0.3 ml) in place of extract was used as the blank. The total antioxidant capacity was expressed as the number of equivalents of Ascorbic acid (AAE).

Results

Phytochemical screening of methanolic extraction of *Ficus benghalensis L* latex
The powder of extract was used to perform various phytochemical tests. It was obtained in extract of *Ficus benghalensis L* latex (shown table no.2).

Total Phenolic content:
The total Phenolic content in extract of *Ficus benghalensis L* latex was get 276±0.84 mg/gm as shown in table no.3.

Total Flavonoids content:
The total Flavonoids content in extract of *Ficus benghalensis L* latex was got 1.84±0.5 mg/gm as shown in table no.3.

DPPH radical scavenging activity:
Free radical scavenging activities of the extract of *Ficus benghalensis L* latex was assessed by the DPPH assay. It was significant decrease in the concentration of DPPH radical due to scavenging potential of the extract. The results show that extract of *Ficus benghalensis L* latex had the highest DPPH scavenging activity with an IC50 value 28.63±0.16 µg/ml., and percentage of DPPH free radical inhibition (fig. 1)

Fecl3 radical scavenging activity:
Free radical scavenging activities of the extract of *Ficus benghalensis L* latex was assessed by the $Fecl_3$ assay. It was significant decrease in the concentration of $Fecl_3$ radical due to scavenging potential of the extract. The results show that extract of *Ficus benghalensis L* latex had the highest Fecl3 scavenging activity with an IC50 value 49.82±1.00 µg/ml., and percentage of Fecl3 free radical inhibition (fig. 2)

Phosphor-molybdenum radical scavenging activity:
Free radical scavenging activities of the extract of *Ficus benghalensis L* latex. seeds was assessed by the phosphor-molybdenum assay. It was significant decrease in the concentration of phospho-molybdenum radical due to scavenging potential of the extract. The results show that extract of *Ficus benghalensis L* latex had the highest phosphor-molybdenum scavenging activity with an IC50 value 31.84±0.12 µg/ml., and percentage of phosphor-molybdenum free radical inhibition (fig.3)

Table no.1. Phytochemical screening of the methanolic extraction of *Ficus benghalensis L* latex

S.No.	***Name of components***	***Name of chemical tests***	***Observation***
1.	**Test of glycosides**		
1.1	Cardiac glycosides	Legal test Keller - Killiani test	Pink colour Reddish-Brow Colour appears at junction of two layers
1.2	Test of anthroquinone glycoside	Brontrager test	Ammonia layer turn pink to red Colour
1.3	Test of cynogegenetic.	Sodium picrate test	Filter paper turns Brink-red.
1.4	Test of Flavonoids	Shinoga test	Pink Colour
1.5	Test of Saponin	Foam and heamolytic test	Persistent foam & heamolytic zone appears
2.	**Test of alkaloids**		
2.1		Dragendraff's test	Orange-brown Ppt
2.2		Wagner test	Reddish-brown Ppt
2.3		Hager test	Yellow Ppt.
2.4		Mayer	Ppt

3.	**Test of Tannins and Phenolic Compounds**		
3.1		5% FeCl3 Solution	Deep blue-black ppt.
3.2		Dil. Iodine Solution	Transient red colour
3.3		Dil. HNO3 Test	Reddish to Yellow colour (+ve)
4.	**Test of protein and amino acid Compounds**		
4.1		Biuret test	Violet colour(+ve)
4.2		Millions test	Red colour(+ve)
4.3	Test of sulpher contain aminoacid.	PbS Ppt	Brownish blue colour(+ve)
4.4	Test of Tyrosine	3 drops millon's reagent	Dark red colour
4.5	Test of Tryptophan	Few drops Glyoxalic acid + conc. H2SO4	reddish ring appeared at junction of two layer
4.6	Test of Glycine	Aq CuSO4 + Fecl3	Red colour
4.7	Test of Cysteine		Black Colour
4.8	Test of Glutamine	CuSO4	Deep blue colour
5.	**Test of Steroid**	Salkowshi reaction	Chloroform layer appear, red and blue
6.	**Test of reducing glycoside**	Benedict's test	Green and red Colour
7.	**Test of non reducing Starch**	Iodine test	Blue colour

Table no. 2. Phytochemical screening of the methanolic extraction of *Ficus benghalensis L* latex:

S. No.	Name of Tests	Results
1.	**Glycosides**	+ve
	Cardiac glycosides	+ve
	Anthroquinone glycoside	+ve
	Cynogegenetic	-ve
	Flavonoids (shinoda test)	+ve
	Coumarin glycoside	-ve
	Saponin glycoside	-ve
2.	***Alkaloids***	+ve
	Dragendraff's test	+ve
	Wagner test	-ve
	Hager test	+ve
	Mayer test	-ve

3.	**Tannins and Phenolic Compound**	+ve
4.	**Proteins**	+ve
	Sulpher contain aminoacid	-ve
	Nihydrin test	+ve
	Biuret test	+ve
	Millions test	-ve
	Tyrosine	-ve
	Tryptophan	-ve
	Glycine	+ve
	Cysteine	-ve
	Glutamine	+ve
5.	Steroid	+ve
6.	Reducing glycoside	+ve

Table no. 3. The total Phenolic and flavonoid content of methanolic extraction of *Ficus benghalensis L* latex:

Extract	Total Phenolic extract (mg GAE/gm)	Total flavonoid content (mg QE/gm)
Ethanolic extract of *Lepidium sativum*	276±0.84	1.84±0.5

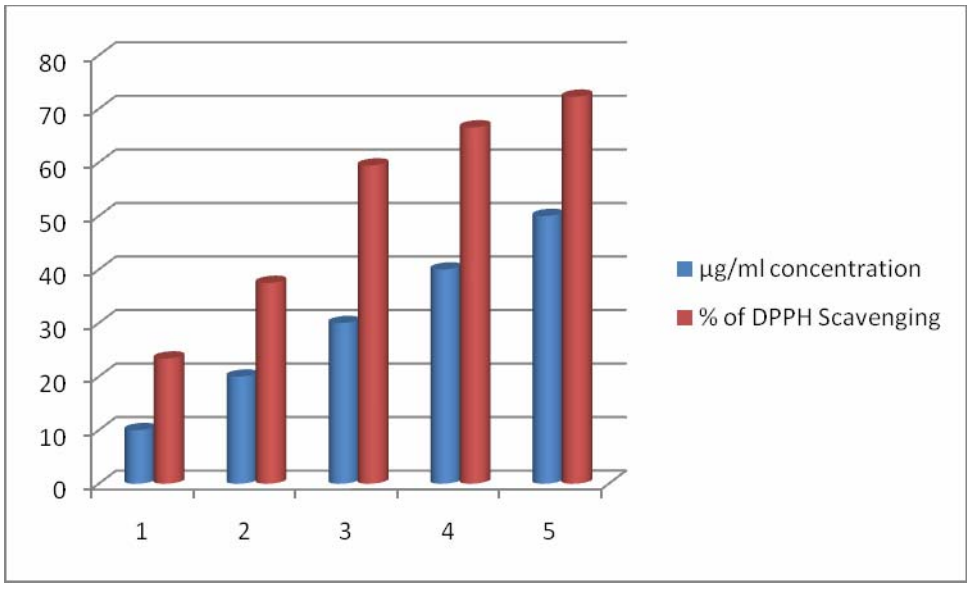

Fig.1 %. DPPH radical scavenging activity of methanolic extraction of *Ficus benghalensis L* latex:

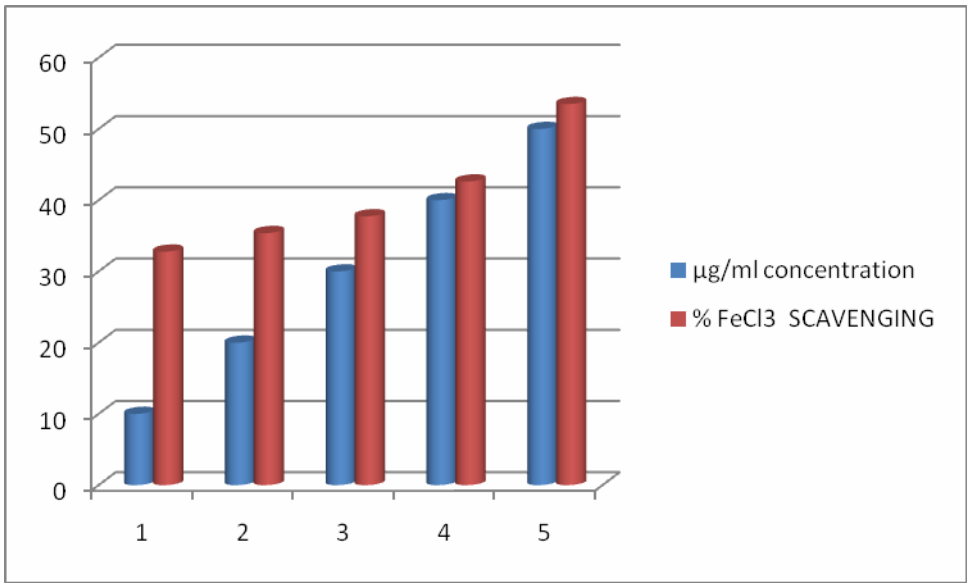

Fig.2. % Fecl3 radical scavenging activity of methanolic extraction of *Ficus benghalensis L* latex:

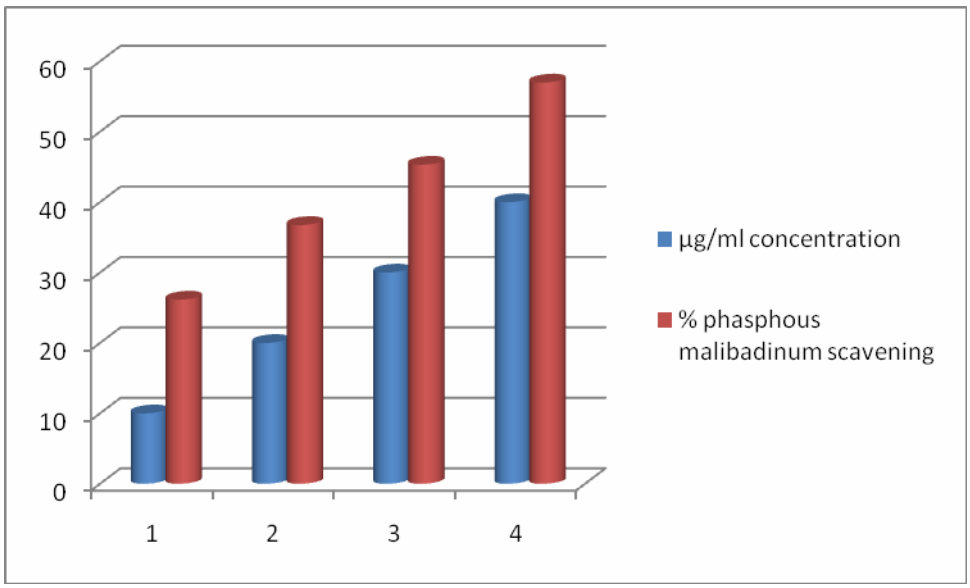

Fig.3. % Phosphor-molybdenum radical scavenging activity of methanolic extraction of *Ficus benghalensis L* latex:

Discussion

The three methods were used for determine the antioxidant activity of the extract of *Ficus benghalensis L* latex Whereas DPPH free radical scavenging was considered a good in- vitro model widely used to assess antioxidant activity within the short time. DPPH was disappear on reduction by antioxidant compound or free radical spices to become stable diagnostic molecules resulting colour change from purple to yellow that can indicates hydrogen denoting ability of extract sample.(22 &23). Our data present study data have lower IC_{50} (28.63±0.16 µg/ml) it was indicated good antioxidant potential of extract. Another present study model of antioxidant assay like Fecl3 and Phosphor-molybdenum in which IC_{50} Value of $fecl_3$ and Phosphor-molybdenum were shown 49.82±1.00 µg/ml and 31.84±0.12 µg/ml. the present study all three method were shown lower IC_{50} Value that indicated that extract have good antioxidant potential due to present phytochemical study of the ethanolic extract have revealed presence of glycoside, alkaloids, tannin (Phenolic compound), Flavonoids. The present antioxidants studies data indicated that methanolic extract of *Ficus benghalensis L* latex may have good antioxidant activity.

Conclusion

The present antioxidants studies data indicated that methanolic extract of *Ficus benghalensis L* latex may have good antioxidant activity.

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