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EVALUATION OF ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITY OF FICUS CARICA (MORACEAE) LINN

Rohtash Singh*[1], Mangal Sain Hooda[1], Neenalchal Trivedi[2] and Krishna Mohan[2]

[1]Janta College of Pharmacy, Butana, Sonipat (Haryana), India.
[2]Teerthanker Mahaveer College of Pharmacy, TMU, Moradabad (U.P).

***Corresponding Author: Rohtash Singh**
Janta College of Pharmacy, Butana, Sonipat (Haryana), India.

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ABSTRACT
Plants used in traditional medicine are potential sources of new biologically active compounds with innumerable therapeutic activities. *Ficus Carica* L. (Moraceae) is such plant used in myths medicines for a number of ailments. The research work to be started with the preparation of successive extract of plant in petroleum ether and ethanol respectively followed by pharmacological investigations including Organoleptic property and qualitative photochemical analysis of leaves of *Ficus Carica* showed the presence of triterpenoids, Glycosides, Alkaloids, Flavonoids, Tannins, Carbohydrates & proteins. The percentage yield values for petroleum ether, chloroform and ethanolic leaves extract were found to be 5.6%, 6.2% & 4.2% respectively. The obtained extract was used to carry out antioxidant study. *Ficus carica* leaves extract exhibited antioxidant activity when subjected to the tests like DPPH, hydrogen peroxide radical scavenging & reducing power assays. The obtained results (IC_{50}) value were for DPPH free radical scavenging assay (2133.61µg/kg), Hydrogen peroxide radical scavenging test (12.93µg/ml), and reducing power assay (974.64µg/ml).

KEYWORDS: Antioxidant, DPPH, Free Radical Scavenging, Anjeer.

INTRODUCTION

1. Free radical scavenging: It is may be designated as molecules sharbs that damage molecules in mitochondria, cell membrane, neurodegenerative disease, DNA (Deoxy Ribose Nucleic acid) & are very unstable, have a tendency to rob electrons from the molecules in the immediate surroundings in order to replace their on losses[1]

1.1 Biological Pathways for Oxygen Reduction[2]

Reactive oxygen species (ROS) produced due to normal Metabolism, Radiation, In-Flammarion, aging, chemical, drugs & so forth.

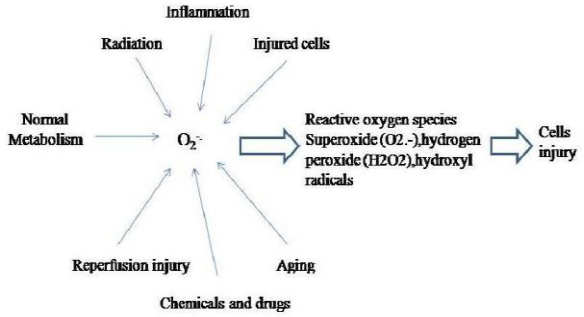


Fig. 1.1: Shown the Biological Pathways for Oxygen Reduction.

2. Antioxidant

Living tissue has a control mechanism to keep reactive oxygen species (ROS) in balance. When ROS are generated in vivo, many antioxidants come into play. Their relative importance depends upon which ROS are generated, how and where are generated, and which target damage is considered. Antioxidants inhibits the production of ROS by direct scavenging, decrease he amounts of oxidants in and around the cells, prevent ROS from reaching their biological targets, limit the propagation of oxidants such as the one that occurs during lipid peroxidation, and oxidative stress, thereby preventing the aging phenomena.[3]

2.1 Type of antioxidants

Antioxidants may be enzymatic and non-enzymatic in nature in which enzymatic system directly or indirectly help in defence against the ROS. (e.g. Superoxide dismutase (SODs remove superoxide by accelerating its conversion into Hydrogen peroxide. SOD enzyme contains manganese (MnSOD), copper and Zinc (CuZnSOD) at its active site in mitochondria and cytosol respectively), Catalase (This enzyme converts H_2O_2 to water and oxygen.). Glutathione peroxidase (In human cells GSHPX is most important H_2O_2 removing enzyme it requires selenium for their action. Non-enzymatic anti-oxidant act as scavenger of ROS and RNS.eg Vitamin E

(Inhibit lipid per oxidation by scavenging Peroxyl radical intermediates), Vitamin C, and Vitamin A, glutathione, uric acid and melatonin (It react with ROS and form disulfide).

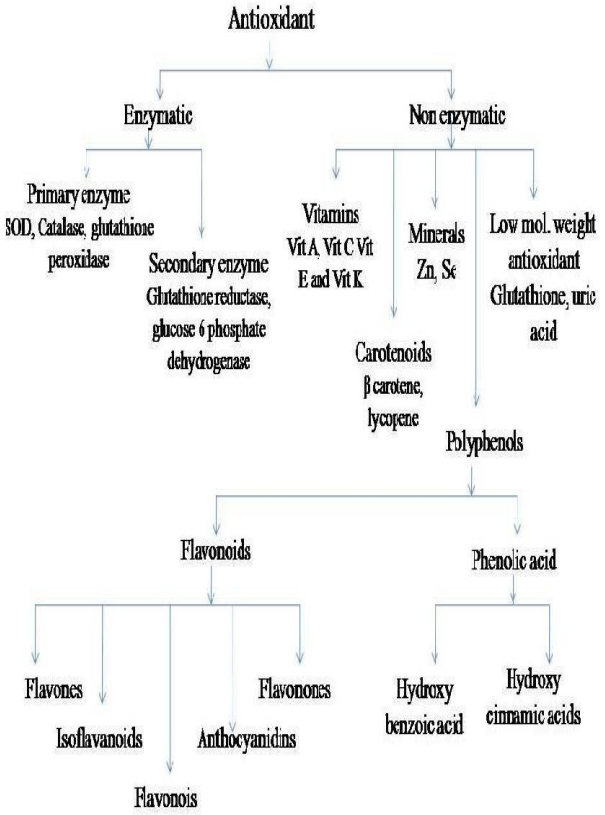


Fig. 1.2: flow chart of antioxidants.

Table 1.1: Various Reactive oxygen species (ROS) and Corresponding Neutralizing Antioxidants.

S. No.	Reactive oxygen species (ROS)	Neutralizing antioxidants
1	Hydroxyl radical	Vitamin C, Glutathione, Flavonoids, Lipoic acid
2	Superoxide	Vitamin C, Gutathione, Flavonoids,
3	Hydrogen peroxide	Vitamin C, Glutathione, beta Carotene, Vitamin E, Coenzyme Q10, Flavonoids, Lipoic acid
4	Lipid peroxides	Beta carotene, vitamin E, Ubiquinone, Flavonoids, Glutathione peroxidase

2.3 Conditions associated with Oxidative Damage & human disease[6,7,8]

Oxidative stress is imbalance between production of reactive species and antioxidant defence leading to several diseases in humans.

• Pancreatitis, Inflammatory bowel disease and colitis, Parkinson's disease, Neonatal lipoprotein oxidation, Drug reactions, Skin lesions, Arthritis and inflammatory diseases, Diabetes.

• Shock, trauma, ischemia, Renal disease andhaemo dialysis, Multiple sclerosis, Aging, Atherosclerosis, Cancer, Pulmonary dysfunction

Factors affecting the efficiency of antioxidants[4,5]

a) Activation energy of antioxidants, b) Oxidation/ reduction potential, c) Solubility, d) pH Stability

MATERIAL AND METHODS

Chemical Details

1, 1-diphenyl-2-piryl hydrazyl (DPPH), Ferric chloride, Ascorbic acid, & ethyl diamine tetra acetic acid (EDTA) were received from Singhla Scientific., Ambala.

Selection and Collection of plant material

The leaves of plant of *Ficus Carica* were selected of the exhaustive literature survey and collected from Meerut, U.P., India in the month of November 2014.

Authentication
The leaves of plant of *Ficus Carica* were authenticated by a senior Botanist Dr. D.C Kasana; head of Department of Botany, I.P College of science, Bulandshahr (U.P), India.

Preparation of Extraction
The powder of the leaf of *Ficus Carica* was extracted according to (Harborne & Baxter., 1995).[9] The powdered drug (around 250 gm) was subjected to continuous hot extraction with the solvents of increasing polarity in soxhlet apparatus, i.e., Petroleum ether (35-40^{0}C), Chloroform & Ethanol. Each time before extracting with the next solvent the plant material was dried in hot air oven at 50^{0}C for one hour. After the effective extraction, the solvent were off the extract were then concentration of water bath for dryness. The obtained extract was stored in refrigerator till any further use.

In-vitro Antioxidant Evaluation: The in-vitro antioxidant properties of leaf extract was determined with reference to DPPH and hydrogen peroxide radical scavenging assay methods.

DPPH free Radical Scavenging Assay[10,11,12]
The DPPH assay of leaf extract was determined by according (Pin Der Duh et. At., 1995)[13] Antioxidants react with DPPH, a stable free radical, which gets reduced to DPPH-H. Consequently, the absorbance gets decreased. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extract in terms of hydrogen donating ability.

Principle: The scavenging reaction between (DPPH) and antioxidant (H-A) can be written as:

(DPPH) + (H-A) ⟶ DPPH-H + (A)
(Purple) (Yellow)

Preparation of Standard Ascorbic acid solution
Various solutions of the ascorbic acid were prepared in 90% methanol to obtain different concentrations (1-100µg/ml). 200µM solution of DPPH (in methanol) was prepared and 1.5ml of this solution was added to 1.5ml of methanolic ascorbic acid solution of different concentrations and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517nm.

Preparation of test solution
Various solutions of leaf extract were prepared in 90% methanol to obtain different concentrations (10-100µg/ml). 200µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5ml of methanolic extract solution of different concentration and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517nm.

Preparation of control solution
For control, 1.5ml of methanol was mixed with 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of the control was taken after 30min against methanol (as blank) at 517 nm. The antioxidant activity of plant leaf extract and ascorbic acid were calculated by using the following formula in terms of % inhibition:

$$\% \text{ Inhibition} = \frac{\text{Ac 230nm} - \text{At 230nm}}{\text{Ac 230nm}} \times 100$$

Where,
Ac = Absorbance of control
At =Absorbance of ascorbic acid/ ethanolic leaf extract.

Hydrogen Peroxide radical Scavenging Assay [14,15]
The ability of the leaf extract to scavenge hydrogen peroxide was determined according to the method of Ruch et.al. (1989)[14] H_2O_2 is a biologically important oxidant. It has very short half-life (1×10^9 at 37º C) that however, restricts its diffusion capability and potency.

Preparation of Standard Ascorbic Acid solution:
Different concentrations of the ascorbic acid were prepared in distilled water to give the solutions of varying concentrations (1-100µg/ml). 1ml of each solution of ascorbic acid was mixed with 2.4ml of 0.1M phosphate buffer and 600µl of 40mM H_2O_2 solutions. After 10 minutes absorbance of different samples were taken at 230nm using phosphate buffer as blank.

Preparation of test solution
Various concentrations of the leaf extracts were prepared in distilled water to give solutions of varying concentrations (1-100µg/ml). 1ml of each solution of plant leaf extract was mixed with 2.4ml of 0.1M phosphate buffer and 600µl of 40mM H_2O_2 solutions. After 10 minutes absorbance of different samples were taken at 230nm using phosphate buffer as blank.

Preparation of Control Solution
For control, 2.5 ml of 0.1M phosphate buffer solution was mixed with 600µl of 40mM H_2O_2 solution. After 10 minutes absorbance of control was taken at 230nm.

Percentage hydrogen peroxide radical scavenging activity of plant leaf extract and ascorbic acid were calculated by using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Ac 230nm} - \text{At 230nm}}{\text{Ac 230nm}} \times 100$$

Where,
Ac = Absorbance of control (0.1M phosphate buffer solution and H_2O_2)
At = Absorbance of ascorbic acid / ethanolic leaf extract

Reducing Power Assay[16]

The reducing power of leaf extract was determined by the method of Yen et.al (1993).[16] The procedure involved the UV- spectrophotometric determination. Two solutions i.e. Standard and Test were prepared.

Preparation of Standard Ascorbic Acid solution

Different concentrations of ascorbic acid were prepared in distilled water to give various concentrations (10-100µg/ml). 1 ml of each concentration of ascorbic acid solutions was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% Potassium ferricyanide solution. The reaction mixture was incubated for 20 min at 50°C. Afterwards 2.5 ml of 10% trichloroacetic acid solution were added and centrifuged at 3000 rpm for 10 min. After separation, 2.5ml of upper layer of each solution was mixed with 2.5 ml of distilled water and 1 ml of 0.1% Ferric chloride (freshly prepared solution).Absorbance was recorded for each solution of ascorbic acid against (0.2M, pH 6.6) phosphate buffer (as blank) at 700nm.

Preparation of test solution

Different solutions of leaf extract were prepared in distilled water to give various concentrations (10-100µg/ml). 1 ml of each solution of leaf extract was mixed with 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of 1% Potassium ferricyanide solution. The reaction mixture was incubated for 20 min at 50^0C. Afterwards 2.5 ml of 10% trichloroacetic acid solution was added and centrifuged at 3000 rpm for 10 min. After separation 2.5 ml of upper layer of each solution was mixed with 2.5ml of distilled water and 1 ml of 0.1% Ferric chloride (freshly prepared solution). Absorbance was recorded for each solution of leaf extract against (0.2M, pH6.6) phosphate buffer (as blank) at 700nm.

The experiment was performed in triplicate. The higher absorbance value indicated greater reducing power of sample.

RESULT AND DISCUSSION

DPPH Free Radical Scavenging Assay

DPPH (1, 1 diphenyl-2-picryl-hydrazyl) assay is widely used to assess antioxidant activities in a relatively short time. DPPH is a stable free radical and accepts an electron or hydrogen radical to burn into a stable diamagnetic molecule.

DPPH assay for *Ficus Carica* was performed by using Ascorbic acid solution as standard. The absorbance data were recorded against the selected concentrations (10-100 µg/ml for ascorbic acid and 10- 100µg/ml for *ficus carica* leaf extract) at 517nm.

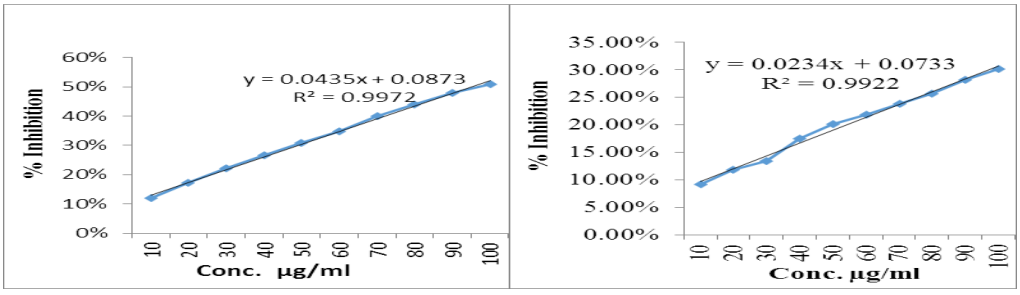
The percentage (%) inhibition curves for DPPH free radical scavenging assay of ascorbic acid and leaf extract were plotted from which IC_{50} values of percentage inhibition of DPPH by ascorbic acid and leaf extract were calculated using regression equations.

Table 1: % Inhibition data of DPPH free radical scavenging assay by ascorbic acid.

S. No	Conc. (µg/ml)	Absorbance (control), Ac	Absorbance (Test), At	% Inhibition
1.	10	0.750	0.660	12.00%
2.	20		0.620	17.33%
3.	30		0.584	22.13%
4.	40		0.550	26.66%
5.	50		0.518	30.93%
6.	60		0.489	34.80%
7.	70		0.450	40.00%
8.	80		0.420	44.00%
9.	90		0.390	48.00%
10.	100		0.368	50.99%

Table 2: % Inhibition data of DPPH free radical scavenging by ethanolic extract of leaves of *Ficus Carica*.

S. No	Conc. µg/ml	Absorbance (control) Ac	Absorbance (Test) At	% Inhibition
1.	10	0.750	0.681	09.20%
2.	20		0.661	11.20%
3.	30		0.649	13.46%
4.	40		0.620	17.46%
5.	50		0.602	20.13%
6.	60		0.586	21.86%
7.	70		0.571	23.86%
8.	80		0.565	25.66%
9.	90		0.538	28.26%
10.	100		0.530	30.22%



(a) **(b)**

Fig. (a): Standard curve of ascorbic acid. Fig. (b): DPPH inhibition curve of leaves extract.

Table 3: IC_{50} value of ascorbic acid and ethanolic extract of *Ficus Carica.*

S. No.	Sample	IC_{50}
1.	Ascorbic acid	1147.41 µg/ml
2.	Leaf extract	2133.61 µg/ml

It was observed that leaf extract extracted significant activity in DPPH assay in the concentration range of 10-100µg/ml. IC_{50} for ascorbic acid was found to be 1147.41µg/ml while for *Ficus Carica* leaf extract as 2133.61µg/ml.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide radical assay is a method used to assess antioxidant activities in a relatively short time. Hydrogen peroxide radical scavenging of *Ficus Carica* leaves extract was estimated by using ascorbic acid solution as standard. The absorbance data were recorded against the selected concentration (10-10µg/ml for ascorbic acid & *Ficus Carica* leaf extract).

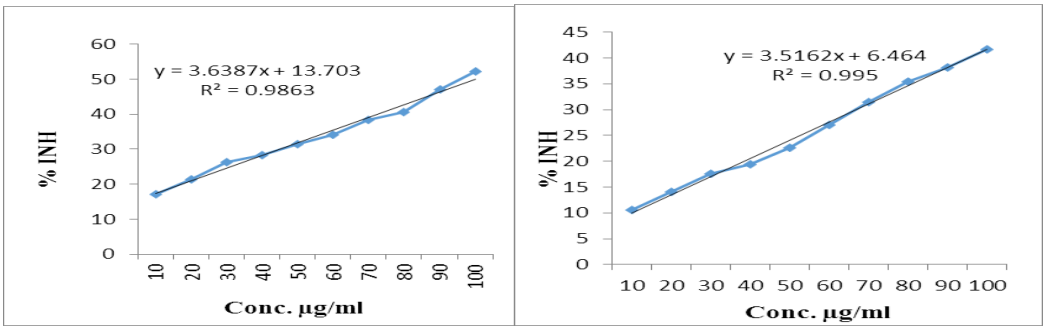
The standard curve for hydrogen peroxide radical scavenging of ascorbic acid and inhibition curve of *Ficus Carica* leaf extract were plotted from which IC_{50} values of percentage inhibition of hydrogen peroxide radical scavenging of ascorbic acid and leaf extract were calculated using regression equations.

Table 4: % Inhibition of hydrogen peroxide radical scavenging by ascorbic acid.

S. No.	Conc.(µg/ml)	Absorbance (Control), Ac	Absorbance (Test), Ac	% Inhibition
1.	10	0.570	0.472	17.19%
2.	20		0.448	21.40%
3.	30		0.420	26.31%
4.	40		0.409	28.24%
5.	50		0.391	31.40%
6.	60		0.375	34.21%
7.	70		0.351	38.42%
8.	80		0338	40.70%
9.	90		0.302	47.01%
10.	100		0.272	52.28%

Table 5: % Inhibition of hydrogen peroxide radical scavenging with ethanolic leaf extract of *Ficus Carica.*

S. No.	Conc.(µg/ml)	Absorbance (Control), Ac	Absorbance (Test), Ac	% Inhibition
1.	10	0.570	0.510	10.52%
2.	20		0.492	14.03%
4.	40		0.459	19.47%
5.	50		0.441	22.63%
6.	60		0.415	27.02%
7.	70		0.391	31.40%
8.	80		0368	35.43%
9.	90		0.352	38.24%
10.	100		0.339	41.75%



(a) **(b)**

Fig. (a): Representing % Inhibition curve & regression curve of hydrogen peroxide radical scavenging by *using* ascorbic acid.

Fig. (b): Representing % Inhibition curve & regression curve of ethanolic extract *Ficus Carica* leaf by hydrogen peroxide radical scavenging by ascorbic acid.

IC_{50} value was calculated by using straight line equations. In hydrogen peroxide scavenging assay, it was observed that extract served as a good scavenger of hydrogen peroxide in the concentration range of 10-100µg/ml. IC_{50} for ascorbic acid was found to be 6.91 µg/ml while that for ficus carica leaf extract it was found to be 12.36 µg/ml.

Table 6: IC_{50} of ascorbic acid and ethanolic leaf extract of *Ficus Carica*

S. No.	Sample	IC_{50}
1.	Ascorbic acid	6.91µg/ml
2.	Leaf extract	12.93 µg/ml

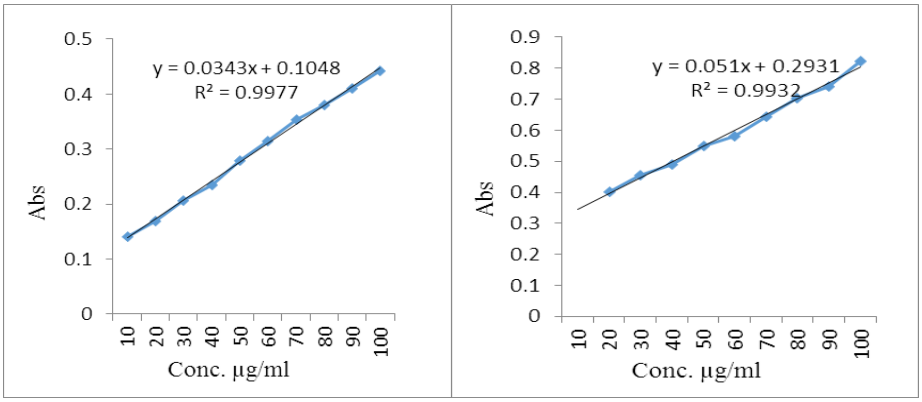
Hydrogen peroxide is a strong oxidizing agent and can inactive few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly. Once entered the cell, H_2O_2 can react with Fe^{+2}/Cu^{2+} to form hydroxyl radical leading to the origin of many of its toxic effects.

Reducing Power Assay

Reducing power of *ficus carica* leaf extract was estimated by using ascorbic acid solution as standard. The absorbance's data were recorded against the selected the concentration curve of ascorbic acid and that of leaf extract were plotted. The y & R^2 values obtained in both case were comparatively studied to determine the reducing power of the leaf extract.

Table 7: Abs. vs Conc. of data of ascorbic acid and ethanolic leaf extract of *Ficus Carica.*

S. No.	Concentration (µg/ml)	Absorbance of ascorbic acid	Absorbance of 95% ethanolic extract
1.	10	0.141	0.331
2.	20	0.170	0.402
3.	30	0.206	0.456
4.	40	0.235	0.491
5.	50	0.279	0.550
6.	60	0.315	0.582
7.	70	0.354	0.645
8.	80	0.380	0.703
9.	90	0.410	0.741
10.	100	0.442	0.824



(a) **(b)**

Fig. (a): Absorbance vs Concentration curve standard of ascorbic acid.
Fig. (b): Absorbance vs Concentration curve standard of 95% ethanolic leaf extract.

Table 8: (IC_{50}) of Absorbance vs Concentration of data of ascorbic acid & ethanolic leaf extract of *Ficus Carica.*

S. No.	Sample	IC_{50}
1.	Ascorbic acid	149.25µg/ml
2.	Leaf extract	974.64µg/ml

The study conclusively depicted that reducing of ascorbic acid higher than *Ficus Carica* leaf extract. The data suggested that the extract was able to donate electrons to reactive radicals converting them into more stable. In this experiment absorbance increased as the concentration enhanced.

CONCLUSION

The present study was carried out to investigate the antioxidant activity of ethanolic extract of *Ficus Carica.* The ethanolic leaves extract were exhibited better antioxidant activity using hydrogen peroxide, DPPH radical scavenging, & reducing power assay. The Anti-oxidant property of the plant can be used in the treatment of different type of disease.

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REFERENCE

1. Halliwell B. The wanderings of a free radical. Free Radic Biol Med, 46: 531-542.
2. Keshri kumar Anand *et a.l* "oxidative stress" the international journal of science & technoledge, (July), 2015; 3.
3. Pouillot A., Polla L.L., Tacchini P., Neequaye A., Polla B. "formulating, Packaging and Marketing of Natural Cosmatic Products, Natural Antioxidants & Their Effects on the Skin" John wiley & Sons Inc., 241-243.
4. Vertuani S, Angusti A, Manfredini S "The antioxidants and proantioxidants network: an overview" Curr Pharma Des, 10: 1677-1694.
5. Kibanova D, Nieto-Camacho A, Cervini-Silva J "Lipid Peroxidation Induced by Expandable Clay Minerals. Environ" Sci. Technol, 43: 7550-7555.
6. Halliwell, B "Free Radicals, Antioxidants, and Human Disease: Curiosity, Cause, or Consequence" *Lancet*, 1994; 344: 721-724.
7. Hennekens, C.H. and Gaziano, J.M., "Antioxidants and Heart Disease: Epidemiology and Clinical Evidence" *Clin Cardiol*, 1993; 16(suppl I): I-10, I-15.
8. Block, G. et al. "Fruit, Vegetables, and Cancer Prevention" A Review of the Epidemiological Evidence, *Nutr Cancer*, 1992; 18(1): 1-29.
9. Harbone, S.B., Baxter, H. Photochemical Dictionary. A handbook of bioactive compounds from plants Taylor and Francis, London.
10. Duh P.D., Yen G.C. "Antioxidative activity of three herbal water extract" food Chemistry, 1997; 60(4): 639-645.
11. Flogel A., Kim D., Chung S., Koo S.I., and Chum O.K. "Journal comparision of ABTS/DPPH Assay to Measure Antioxidant Capacity In Popular Antioxidant- Rich Foods" Food Comparison and Analysis, 2011; 24: 1043-1048.
12. Brand Williams W., Cuvelier M.E., and Berset C. "Use of a Free Radical Method to Evaluate Antioxidant Activity" Lebennsm-Aiss. U.-Technol, 1995; 28: 25-30.
13. Li C, Bu PB, Yue DK, Sun YF. "Chemical constituents from the roots of *Ficus Carica Zhongguo Zhong Yao ZaZhi*, 2006; 31: 131-133.
14. Muthukumaran P., Shanmuganathan P., and Malathi C. "In vitro Antioxidant Evaluation of Mimosa Pudica" Asian J. Pharma. Res., 1: 44-46.
15. Jayprakasha G.K., Rao L.J., and Sakaroah K.K. "Antioxidant Activities of Flavidin in different In-Vitro model systems" Bioorganic and Medicinal chemistry, 12: 5141-5146.
16. Yu L., Zhao M., Wang J.S., Cui C., Yang B., Jiang Y. And Zhao Q. "Antioxidant, Immunomodulatory & anti-breast cancer activities of phenolic extract from pine (Pinus massoniana L.) bark" Innovative food and Science & emerging Technologies, 9: 122-12.