Phytochemical Screening and In Vitro Free Radical Scavenging Activity of Aqueous Extract of *Ficus Species*

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Abstract*—The genus *Ficus*, widely distributed across tropical and subtropical regions, has long been recognized for its therapeutic potential in traditional medicine. This study aimed to evaluate the phytochemical composition and in vitro antioxidant activity of aqueous extracts from selected *Ficus* species. Qualitative phytochemical screening revealed the presence of bioactive constituents such as flavonoids, tannins, saponins, phenolics, and glycosides, indicating their potential pharmacological importance. The antioxidant capacity was assessed using in vitro free radical scavenging assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) methods. Results demonstrated a dose-dependent increase in radical scavenging activity, with IC_{50} values suggesting significant antioxidant potential, particularly in *Ficus racemosa* and *Ficus benghalensis* extracts. The observed bioactivity is attributed to the presence of polyphenolic compounds known to neutralize reactive oxygen species and mitigate oxidative stress-related cellular damage. These findings support the traditional use of *Ficus* species and provide a scientific basis for their role as natural antioxidants. Further research, including compound isolation and in vivo studies, is warranted to fully understand the mechanisms involved and to explore therapeutic applications. The study contributes valuable insights into the phytochemical richness and antioxidant potential of *Ficus* species, underscoring their relevance in developing plant-based health interventions and natural antioxidant formulations.

Keywords— Antioxidant activity, Aqueous extract, ABTS assay, DPPH assay, *Ficus* species, Phytochemical screening etc.

I.INTRODUCTION

An antioxidant is a molecule that can inhibit the oxidation of other molecules. Oxidation refers to a chemical reaction in which electrons are transferred from a substance to an oxidising agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. They do this by being oxidised themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols.[1]
The aim of current research work is the study of potency of natural herbal plants as anti-oxidant agents by exploring their pharmacological and phytochemical properties. This study mainly emphasizes the role of natural herbal plants since they are the precious resources for food and medicine in human life.[2] To attain the goal of our research, we have selected the herbal plant such as *Ficus benghalensis* belongs to the family *Moraceae* of plant kingdom. We have selected this plant for present research since it is rich in flavonoids and other polyphenolic compounds.[3]

II. MATERIALS AND METHODS

2.1. Plant materials:

The plant material such as aerial roots of *Ficus benghalensis* were freshly collected from nearby villages of Rajampet of Andhra Pradesh state. The plant is authenticated by Dr. J. Kamakshamma, Professor and Head, Department of Botany, S.V. University, Tirupathi. Voucher specimens (2023/642) of the plants were deposited in a college.

2.2. Solvents, Chemical reagents and Equipments:

The solvents used in the present work were pet ether, chloroform and double distilled water. All the glass ware used in the study was made by Borosil and equipments were manufactured by Systronics Pvt, Ltd. In the present study different glass ware and equipment were used they are; Electric water bath, Rotavapor Buchi R-114,Digital balance, Weighing bottle, Dessicator, 40 Mesh sieve, Mixer grinder, Muffle furnace, Hot air oven, Conical flask,

Compound microscope, Capillary tubes, Aspirator, Funnel, Inoculating loop, Petri dishes.

2.3. Preparation of Extracts:

The freshly collected plant material was washed, shadow dried and then dried in hot air oven at a temperature not more than 50°C. The dried material was coarsely powdered using an electric blender. Powdered material (500g) was then packed in soxhlet apparatus and successively extracted with pet ether, chloroform and distilled water. Finally extracts were concentrated in rotary evaporator at a temperature not more than 50°C and then, dried under vacuum dessicator. The dried extract thus obtained was used for further experiments. In the current research we have used the aqueous extract of aerial roots of *Ficus benghalensis* plant.[4]

2.4. Preliminary phytochemical screening:

Preliminary phytochemical screening was done using the specified protocols for the qualitative analysis of Alkaloids, carbohydrates, fixed oils, flavonoids, glycosides, phyto sterol/terpenes, saponins, and tannins/phenols.[5]

2.5. Ferric reducing antioxidant power assay:

The reducing power of extracts was determined by the method of gulcin. Briefly, an aliquot of 0.1ml of extracts at various concentrations was mixed with phosphate buffer [2.5ml, 0.2 M, pH 6.6] and potassium ferric cyanide [2.5ml, 1% w/v, in water]. The mixture was incubated at 50^0C for 20 min and the reaction was stopped by addition of 2.5 ml of trichloroacetic acid [10% w/v, in water], followed by centrifugation in 3000rpm for 10 min. The upper layer of solution [2.5ml] was mixed with distilled water [2.5ml] and $FeCl_3$ [0.5 ml, 0.1% w/v, in water], and the absorbance was measured at 700nm against blanks that contained all except the sample extracts.[6]

2.6. Hydrogen peroxide scavenging activity:

The hydrogen peroxide scavenging activity of MLS and CELS were determined. A solution of H_2O_2 [40mM] was prepared buffer [pH 7.4]. 1ml of different concentrations of sample was added to a H_2O_2 solution [0.6ml, 40mM]. The absorbance value of the reaction mixture was recorded at 230nm. Blank solution was containing the phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging of samples and standard compounds was calculated as % Scavenging $H_2O_2 = [A_o\text{-}A_1]/A_o \times 100$ Where A_0 is the absorbance of the control, and A_1 is the absorbance in the presence of the sample or standards.[7]

2.7. Free radical scavenging activity (DPPH)

The free radical scavenging activity of aqueous extract of *ficus benghalensis* was measured by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method of Blois (1958). 0.2mM solution of DPPH in methanol was prepared and 100µl of this solution was added to various concentrations of aqueous extracts at the concentrations of 10, 20, 30, 40 and 50µg/ml. After 30 minutes, absorbance was measured at 517nm. Butylated hydroxytoluene (BHT) was used as the reference material. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples.[8]

Percentage of inhibition = Abs control-Abs test x 100

2.8. Nitric oxide [NO] scavenging activity:

Nitric oxide is a modulator of physiological and pathological functions in cardio vascular neuronal and immune system it is involved as aninter cellular signal and defensive cytotoxic in nervous cardio vascular and immune system. Sodium nitroprusside 5mg in phosphate buffer pH 7.4 was incubated with 100µm concentration of drug dissolved in suitable solvents [dioxine/methanol] and tubes were incubated at 25^0C for 120min. Control experiment was kept without test sample but equal amount of solvent was added in an identical manner. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5ml with Griess reagent.[9]

III. RESULTS

3.1. Extraction process:

The percentage yield of extracts of *Ficus benghalensis* aerial roots was determined and was represented in the following table.

Table 1: Percentage yields of *Ficus benghalensis aerial root extract.*

Extracts	% yield of *Ficus benghalensis*
Petroleum ether	3.1
Chloroform	3.4
Distilled water	13.4

3.2. Preliminary phytochemical Studies Results:

The Experimental results of preliminary phytochemical studies on *Ficus benghalensis* aerial roots was tabulated in Tab.2

Table 2: Preliminary phytochemical screening of *Ficus benghalensis* aerial roots.

Type of phyto chemical constituents	Aqueous extract of aerial roots of *Ficus benghalensis*
Alkaloid	-
Carbohydrates	+
Glycosides	-
Saponin glycosides	+
Proteins	+
Volatile oils	+
Fats and fixed oils	-
Steroids	+
Flavonoids	+

3.3. Anti-oxidant activity studies:

3.3.1: Ferric reducing anti oxidant power assay:

The percentage of Ferric reducing anti oxidant power assay of ethanolic extract of *Ficus benghalensis* presented in Table 3. The aqueous extract of *Ficus benghalensis* exhibited a maximum Ferric reducing anti oxidant power assay 136.23% at 50µg/ml where as for Ascorbic acid standard it was found to be 149.27% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 6µg/ml and 5µg/ml respectively.

Table 3: Results of Ferric reducing anti oxidant power assay of *Ficus benghalensis.*

S.No	Conc.(µg/ml)	Percentage inhibition of Activity	
		Sample (*Ficus benghalensis*)	Standard (Ascorbic acid)
1.	10	81.159%	98.550%
2.	20	89.130%	108.69%
3.	30	100%	111.59%
4.	40	105.79%	132.60%
5.	50	136.23%	149.27%
6.	IC 50	6	5

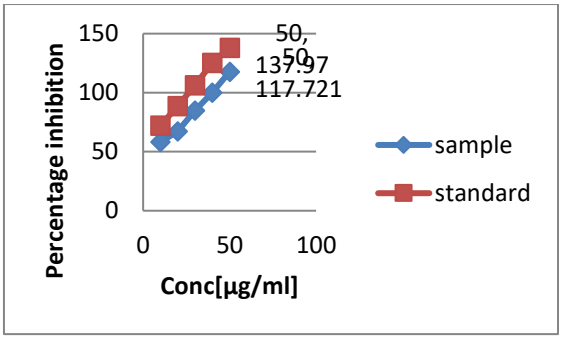

Fig 1: Results of Ferric reducing anti oxidant power [FRAP] assay

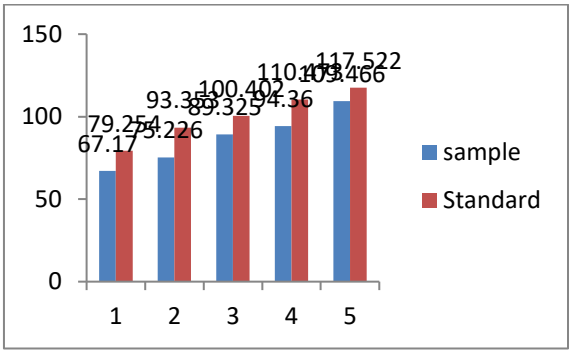

Fig 2: Results of Ferric reducing anti oxidant power assay

3.3.2. Hydrogen Peroxide scavenging Assay:

The percentage of Hydrogen Peroxide scavenging of aqueous extract of *Ficus benghalensis* presented in Table 4. The aqueous extract of *Ficus benghalensis* exhibited a maximum Hydrogen Peroxide scavenging activity 117.721% at 50µg/ml where as for Ascorbic acid standard it was found to be 137.974% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 8µg/ml and 6µg/ml respectively.

Table 4: Hydrogen Peroxide scavenging assay of *Ficus benghalensis.*

S.No	Conc. (µg/ml)	Percentage inhibition of Activity	
		Sample (*Ficus benghalensis*)	Standard (Ascorbic acid)
1.	10	58.227%	72.151%
2.	20	67.088%	88.607%
3.	30	84.810%	106.32%
4.	40	100%	125.31%
5.	50	117.721%	137.974%
6.	IC 50	8	6

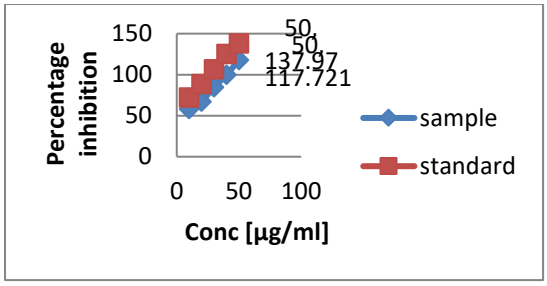

Fig 3: Results of Hydrogen peroxide radical scavenging activity:

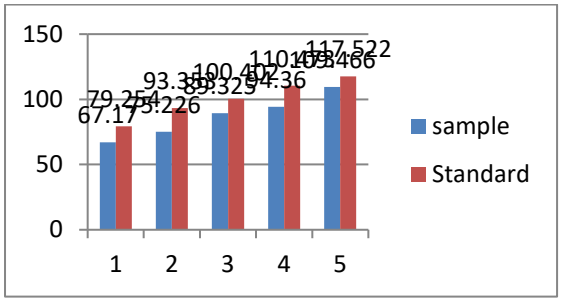

Fig 4: Results of Hydrogen peroxide radical scavenging activity:

3.3.3. DPPH scavenging activity:

The percentage of DPPH radical scavenging activity of aqueous extract of *Ficus benghalensis* presented in Table 5. The aqueous extract of *Ficus benghalensis* exhibited a maximum DPPH scavenging activity 109.466% at 50µg/ml where as for Ascorbic acid standard it was found to be 117.522% at 50µg/ml. The IC50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 6µg/ml and 6µg/ml respectively.

Table 5: DPPH free radical scavenging activity of *Ficus benghalensis*.

S.No	Conc. (µg/ml)	Percentage inhibition of Activity	
		Sample (*Ficus benghalensis*)	Standard (Ascorbic acid)
1.	10	67.170%	79.254%
2.	20	75.226%	93.353%
3.	30	89.325%	100.402%
4.	40	94.360%	110.473%
5.	50	109.466%	117.522%
6.	IC 50	6	6

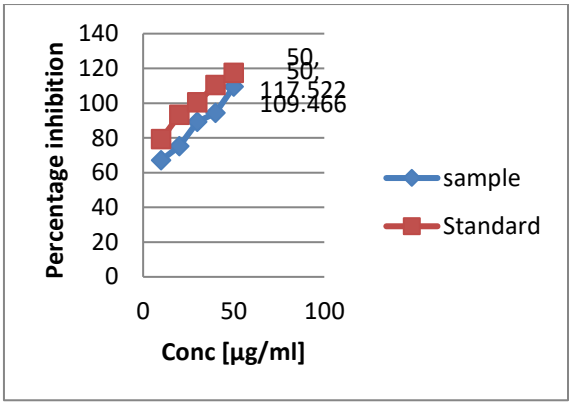

Fig 5: Results of DPPH free radical scavenging activity

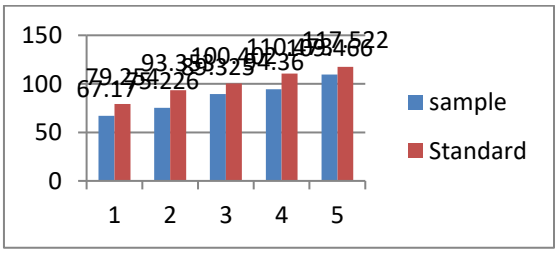

Fig 6: Results of DPPH free radical scavenging activity

3.3.4. Nitric oxide scavenging activity:

The percentage of Nitric oxide radical scavenging activity of aqueous extract of *Ficus benghalensis* presented in Table 6. The aqueous extract of *Ficus benghalensis* exhibited a maximum Nitric oxide scavenging activity 117.692% at 50µg/ml where as for Ascorbic acid standard it was found to be 124.615% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 6µg/ml and 6µg/ml respectively.

Table 6: Results of Nitric oxide scavenging activity of *Ficus benghalensis.*

S.No	Conc.(µg/ml)	Percentage inhibition of Activity	
		Ficus benghalensis	Standard(Ascorbic acid)
1.	10	79.230 %	86.153%
2.	20	89.232%	102.307%
3.	30	98.461%	113.846%
4.	40	109.210%	120%
5.	50	117.492%	124.615%

6.	IC 50	6	6

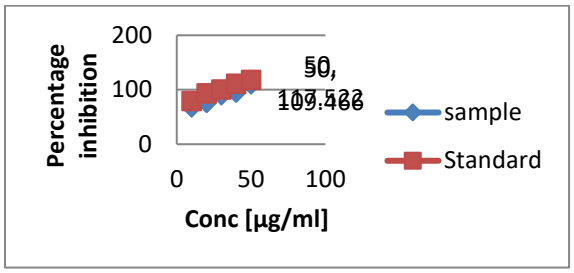

Fig 7: Results of Nitric oxide radical scavenging activity

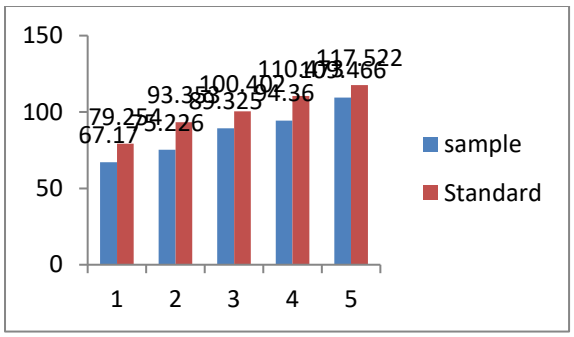

Fig 8: Results of Nitric oxide radical scavenging activity

IV. DISCUSSION AND CONCLUSION

The extraction process of *Ficus benghalensis* aerial roots has revealed that, *Ficus benghalensis* has given higher yield in distilled water (aqueous) rather than petrollium ether and chloroform.[10] Hence aqueous extract was selected for the further studies. By using the aqueous extract preliminary phytochemical studies was performed and the results of these studies have revealed that aqueous extract weas rich in phenolic and poly phenolic compounds and particularly flavonoids. As the extract was rich in flavanoids, we furtherly performed antioxidant activitiy.[11] The anti oxidant activity of aqueous extract of *Ficus benghalensis* aerial roots was performed and the results have shown that our extrcthas is having good potency as anti oxidant agent at the concentration of 50µg/mL in contrast to the standard Ascorbic acid in various methods.

In Ferric reducing anti oxidant power Assay, Ferric reducing anti oxidant power of our extract is 136.23% at 50µg/ml where as for Ascorbic acid standard it was found to be 149.27% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 6µg/ml and 5µg/ml respectively. In Hydrogen peroxide scavenging activity, The aqueous extract of *Ficus benghalensis* exhibited a maximum Hydrogen Peroxide scavenging activity i,e 117.721% at 50µg/ml where as for Ascorbic acid standard it was found to be 137.974% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 8µg/ml and 6µg/ml respectively.In DPPH scavenging assay our aqueous extract has exhibited a maximum DPPH scavenging activity i,e 109.466% at 50µg/ml where as for Ascorbic acid standard it was found to be 117.522% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 6µg/ml and 6µg/ml respectively. In Nitric oxide scavenging activity our aqueous extract has exhibited a maximum Nitric oxide scavenging activity i,e 117.692% at 50µg/ml where as for Ascorbic acid standard it was found to be 124.615% at 50µg/ml. The IC 50 values of the aqueous extract of *Ficus benghalensis* and Ascorbic acid was found to be 6µg/ml and 6µg/ml respectively.

V. CONCLUSION

The present study highlights the significant phytochemical constituents and potent antioxidant activity of the aqueous extract of *Ficus* species. The qualitative phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, phenols, tannins, and saponins, which are known to contribute to free radical scavenging. The in vitro antioxidant assays demonstrated that the *Ficus* extract possesses considerable free radical scavenging potential, indicating its therapeutic promise in combating oxidative stress-related diseases. These findings support the traditional use of *Ficus* species in herbal medicine and suggest further investigation, including quantitative analysis, compound isolation, and in vivo studies, to explore its full pharmacological potential and clinical relevance.

REFERNCCES

[1] Jain Saloni Jayeshkumar *et.al*, Phytoconstituents from aerial roots of *Ficus benghalensis* linn, *Indo American Journal of Pharmaceutical Research*, 2015 ISSN NO: 2231-6876.

[2] Kumar Hement *et al.*, Evaluation of *In-Vitro* Antioxidant Activity in *Ficus religiosa* (L.) Leaves, *International journal of research in pharmacy and science*, 2011, 1(2), 102-110.
[3] Harborne, J.B., Phytochemical Methods, A guide to modern techniques of Plant analysis. 2nd edn, London, New York. 1973: p 90.
[4] Swaran J.S. Flora, Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure, Oxidative Medicine and Cellular Longevity, 2(4),2009, 191-206.
[5] Asmaa Missoum, An update review on *Hibiscus rosa sinensis* phytochemistry and medicinal uses, *Journal of Ayurvedic and Herbal Medicine*, 4(3), (2018) 135-146.
[6] Lokesh J Shetty, Farouk M Sakr, Kais Al-Obaidy, Mohammed J Patel, Hidayatullah Shareef, A brief review on medicinal plant *Tagetes erecta Linn*, Journal of Applied Pharmaceutical Science, 5 (3), 2015, 091-095.
[7] Khaloud KA, Hossain MA. New prenylated flavonoids from the leaves of *Dodonea viscosa* native to the Sultanate of Oman. Pac Sci Rev A: *Nat Sci Eng* 2016; 18:53–61.
[8] Koleva II et al., Screening of plant extracts for antioxidant activity, a comparitive study on three testing method, *Phytochemical analysis*, 13, 2002, 8-17.
[9] Ain VC et al, Antioxidant and antimicrobial activities of *Alangium salvifolium* (lF) Wang root. *Global J Pharm*, 2010;4 (1):13–8.
[10] Kolavali yalla reddy et al. "Antioxidant properties of methanolic extract of Oxalis corniculata." *International Journal of Phytopharmacology*, 1.1 (2010): 43-46.
[11] Malta, R.H. Liu, Analyses of Total Phenolics, Total Flavonoids, and Total Antioxidant Activities in Foods and Dietary Supplements, *Encyclopedia of Agriculture and Food Systems*, 2014, 305-314.