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Original Article

In-vitro Antioxidant and Anti-inflammatory Potential of *Ficus infectoria* Fruits

Prince Ahad Mir[1,*], Shreya Mahajan[2], Ashish Verma[2], Nishant Kumar[3], Manisha Arora[4], Navneet Nagpal[5]

ABSTRACT

Background: Antioxidants are chemical substances, either synthetic or natural, that can avert or slow down a range of cellular damage. **Objectives:** The purpose of this study was to verify the antioxidant and anti-inflammatory properties of alcoholic and aqueous extracts of *Ficus infectoria* fruits. **Materials and Methods:** DPPH free radical quenching test, reducing power technique, and hydrogen peroxide scavenging tests were used to assess antioxidant efficacy. *In vitro* anti-inflammatory experiments were also investigated for membrane stabilization potential and inhibition of proteins from denaturation. **Results:** Fruits included alkaloids, steroids, phenolics, flavonoids, and amino acids, according to phytochemical study. The total phenolic and flavonoid content of the methanolic and aqueous preparations were determined to be 576.16 ± 129.10 and 416.12 ± 112.01 mg/g Gallic Acid Equivalents (GAE) and 423.17 ± 56.48 and 253.35 ± 24.07 mg/g Rutin Equivalents (RE) respectively. In comparison to the benchmarks Rutin and ascorbic acid, both preparations showed significant DPPH and H_2O_2 quenching efficacy. As compared to the standard Indomethacin, both preparations have strong membrane stabilising and protein denaturation inhibitory potencies **Conclusion:** The current findings point to *Ficus infectoria*'s antioxidant and anti-inflammatory properties. However, the active ingredients relevant for the activity must be identified and purified, and the exact mechanism must be determined.

Key words: *Ficus infectoria,* Membrane stabilization, Protein denaturation, Antioxidant, Anti-inflammatory.

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INTRODUCTION

Endogenous defence mechanisms such as catalase system, superoxide dismutase, and peroxidase-glutathione scavenge oxygen free radicals produced within human bodies as a result of metabolism and cellular oxidation.[1,2] Excessive free radical production is extremely toxic and contributes to oxidative stress that promotes pathological conditions including hypertension, coronary artery disease, cancer, immunosuppression, ageing, neurological disorders, diabetes, and others.[3] Antioxidants are substances that effectively eliminate and obstruct the oxidation of chain processes, thus preventing or delaying the oxidative degradation of other molecules.[4] Inflammation is just a defence mechanism involving blood vessels, immune cells, and chemical mediators that occurs in response to any illness or stimulation which causes cellular degeneration.[5] The primary goal of the treatment is to begin tissue repair via eliminating necrotic cells and other sources of cell damage.[6] Excessive and persistent inflammation leads to tissue damage, organ failure, and death.[7] The activation of nuclear factors like AP-1 or NFB, which encourage the production of cytokines, by reactive oxygen species generated within the body, plays crucial role mostly in spreading of infection to multiple organs. Endothelial cells triggered via synergistic action of cytokines and free radicals, resulting in the production of adhesion molecules and inflammatory mediators. Furthermore at the site of inflammation, free radicals involve other cellular components leads to loss of function and even death.[8]

Ficus infectoria, often known as Philkan/white Figure, is a member of the Moraceae family. It is an important medicinal plant that is widely spread throughout the world's tropical and subtropical regions. It is a massive deciduous, fast-growing, densely foliaceous tree with a beautifully shaped crown that may reach a height of approximately 20 metres.[9] The bark of the plant has traditionally been used to treat ulcers and leucorrhoea, as well as to get rid of round worms. The leaves are also used to treat a number of skin conditions. The pharmacologically active elements found from the leaves of *Ficus infectoria* include -amyrin, -amyrin, lupeol, stigmasterol, and compesterol. Infectorin, bergapten, scutellarein, bergaptol, scutellarein glucoside and sorbifolin, have also been isolated from the complete plant.[10,11] The anti-oxidant and anti-inflammatory effects of aqueous and alcoholic preparations of *Ficus infectoria* fruits, as well as phytochemical screening, were investigated in this work.

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MATERIALS AND METHODS

Plant material collection and extract preparation

Mature fruits from *Ficus infectoria* were harvested in October 2019 from the Guru Nanak Dev University (GNDU) campus in Amritsar, Punjab. Dr. Jatinder Kaur, Head of Department, Botanical and Environmental Sciences GNDU, validated the plant, and the specimen was preserved in the GNDU herbarium under the voucher number 1793-GNDU. After drying in the shade, the fruits were roughly pulverized. 500 g of powdered drug was treated to a 12-hr hot extraction with methanol using the Soxhlet device. Whatman filtration paper no. 2 was used to filter the extract. Under vacuum, the extract was concentrated. The aqueous extract was made using a simple maceration process.

Procurement of chemicals

All the chemicals utilized were procured from reputable vendors such as Rankem Gurugram, Haryana 122002, Central Drug House (P) Ltd – CDH Ansari Rd, Daryaganj, New Delhi, Delhi 110002.

Antioxidant activity

Determinations of DPPH free radical quenching

With slight modifications, the technique described by Braca *et al.* was used to determine the DPPH free radical quenching capability of alcoholic and aqueous extracts of *Ficus infectoria* fruits. Simply, a freshly made solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was placed in test tubes, followed by various dilutions of fruit extracts (25 g/mL to 125 g/mL). For 30 min, the mixture was left at room temperature. A control sample was generated using a similar process but without the test sample. Using Ascorbic acid as a reference, the absorbance of the incubated solution was measured spectrophotometrically at 517 nm. Methanol was utilised as a blank solution throughout the operation.[12]

Reducing power estimation

The reductive efficacy of said preparations was estimated using Oyaizu's method with minimal modifications. Simply, the test blend was made by combining various dilutions of *Ficus infectoria* fruit extracts (100-500 g) with 0.2 M phosphate buffer (pH 6.6) 2.5 mL, followed by 1 percent potassium ferricyanide 2.5 mL [$K_3Fe(16)N_6$], and incubating for 20 min at 500C before adding 10 percent trichloroacetic acid 2.5 mL. After centrifuging the whole mix at 3000 rpm for 10 min, the top layer of solution (2.5 mL) was sorted off and dissolved in distilled water (2.5 mL), followed by 0.1 percent $FeCl_3$ 0.5 mL. A similar method was used to make standard ascorbic acid. At 700 nm, the absorbance of the solution was determined spectrophotometrically against a blank solution. The enhanced absorbance of said blend is exactly proportional to the reducing power.[13,14]

H_2O_2 Scavenging Potency: The H_2O_2 scavenging potency of *F. infectoria* fruit extracts was computed utilizing Ruch *et al.* method with slight changes. In a 7.4 pH phosphate buffer, a 40 mM H_2O_2 solution was freshly produced. Various dilutions of the fruit extract prepared in distilled water ranging from 250-1250 µg/mL were combined into a 0.6 mL H_2O_2 solution. The absorbance of the blend was determined spectrophotometerically at 230 nm after 10 min, using a blank consisting of phosphate buffer devoid of H_2O_2. The % H_2O_2 scavenged by the test and standard solution was calculated using the following formula:

$$\% \text{ Scavenged } [H_2O_2] = \frac{A_0 - A_1}{A_0} \times 100$$

The absorbance of the control solution is Ao, whereas the absorbance of the test and standard solutions is A1.[15,16]

Total phenolics: The phenolic content in aqueous and alcoholic preparations of *Ficus infectoria* fruits was determined using the Folin–Ciocalteu technique. The extract was dissolved in distilled water to make a stock concentration of 1 mg/mL. In a test tube, 0.5 mL of extract was combined with 2.5 mL of 10% Folin-Ciocalteu's reagent made in distilled water, then 2.5 mL of 7.5 percent $NaHCO_3$. The complete reagents, except the test solution, were mixed to make the blank solution. The absorbance was measured spectrophotometrically at 765 nm after incubating the reaction mixture at 45°C for 45 min. The entire analysis was carried out in triplicate using Gallic acid as a standard, and the findings were represented as mg GAE / gram of dry weight.[17]

Total flavonoids: The flavonoid content of *F. infectoria* fruit extract was determined using Quettier's method. The residue was dissolved in alcohol at a conc. of 1 mg/mL for the estimate. I mL of 2% $AlCl_3$ was added to the methanolic solution. At 370°C, the reaction mixture was incubated for 1 hr. Each test analysis was carried out three times. At 415 nm, the absorbance was measured spectophometrically. The calibration curve was created using Rutin as a reference. The results were expressed in milligrams of rutin equivalents per milligram of dry extract.[18]

Anti-inflammatory potency

The human red blood cell (HRBC) membrane stabilization method: 10 ml of blood were drawn in the heparinized vials from the healthy human subjects who did not have the prior 2 weeks history of the administration of anti-inflammatory drugs. Following that, the blood was centrifuged for ten minutes at 3000 rpm. The packed cells were suspended in an equivalent amount of saline solution after the supernatant was removed. The tubes were centrifuged and washed once more to get the clear supernatant. Dissolving human red blood cells (HRBC) in normal saline yielded a 10% suspension. After then, the suspension was held at 40°C without being disturbed. The test sample was made by combining 0.5 mL of HRBC suspension with different concentrations of fruit extracts in 1 mL of Phosphate buffer (pH 7.4), then adding 2 mL of hypotonic or hypo saline solution. Taking the different concentrations of Indomethacin as a standard, the same suspension was prepared. After incubating all the samples for 30 min at 37°C, the reaction mixtures were centrifuged at 3000 rpm. The supernatant liquid was separated and the content of hemoglobin was determined spectrophotometrically at the wavelength of 560 nm. The % membrane-stabilizing potency was estimated as follows.[19,20]

$$\% \text{ Stabilizing activity} = \frac{\text{optical density of drug}}{\text{optical density of control}} \times 100$$

Table 1: Phytochemical analysis of alcoholic and aqueous extracts *Ficus infectoria*.

Class of compounds	Alcoholic extract	Aqueous Extract
Alkaloids	++	++
Carbohydrates	–	–
Proteins	–	–
Steroids	++	++
Phenolic compounds	++	++
Flavonoids	++	++
Amino acids	++	++
Saponins	–	–
Glycosides	–	–

+ (Present) (–) Absent

Inhibition of protein denaturation: Protein denaturation assay was performed using slightly modified procedure prescribed by Sakat *et al.* 0.2 mL of egg albumin was taken from fresh hen's egg and dissolved in 2.8 ml of phosphate buffer saline of pH 6.4. To this solution, different concentrations of the fruit extract were added and the final volume was adjusted to 5 mL with double distilled water. After incubating the test samples at 37.2°C for 15 min, all of the reaction mixtures were heated for 20 min at 51°C before chilling. Against a blank, the turbidity intensity was calculated to be 660 nm. The extract's % protein denaturation inhibition was then calculated as:[21]

$$\text{Percentage inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

RESULTS AND DISCUSSIONS

Phytochemical analysis of fruits revealed the presence of alkaloids, steroids, phenolics, flavonoids and amino acids Table 1.

DPPH quenching potency

At a concentration of 250 µg/ mL, the capacity of alcoholic and aqueous preparations of *F. infectoria* to scavenge DPPH free radicals was determined as inhibitory activity, which was 90.17 percent and 82.12 percent, respectively, while as rutin had an inhibitory activity of 97.89 percent. Figure 1 and Table 2.

Reducing power

In comparison to ascorbic acid, both the preparations of *F. infectoria* showed good reducing power. Figure 2. At 500 µg/mL, the alcoholic and aqueous preparations had reducing powers of 1.054 and 0.978, respectively. At the very same amount, standard ascorbic acid had a reducing power of 1.529. Table 3.

H_2O_2 Scavenging Potency

The ability of alcoholic and aqueous preparations of *F. infectoria* to scavenge H_2O_2 was assessed as a percentage inhibition of scavenging. At a concentration of 1000 µg/mL, the alcoholic and aqueous preparations inhibited 78.41 percent and 61.25 percent of the enzymes, respectively, comparing to 98.72 percent for normal ascorbic acid. Figure 3 and Table 4.

Total phenolic and flavonoid content: Figure 4 depicts the total phenolic and flavonoid levels of alcoholic and aqueous *F. infectoria* rhizome preparations. Total phenolics in alcoholic and water preparations (mg/g) in Gallic acid equivalent were 576.16 and 416.21 mg/g, respectively, while total flavonoids in alcoholic and aqueous extracts (mg/g) in Rutin Equivalents were 423.17 and 253.35 mg/g (RE) Table 5.

Membrane stabilization

At a dosage of 125 µg/mL, the percentage membrane stabilising efficacy of methanolic and aqueous preparations of *F. infectoria* was 82.09 and 65.47 percent, respectively, while indomethacin exhibited 96.76 percent membrane stabilising potency. Table 6 and Figure 5.

Protein denaturation

Methanolic and aqueous preparations of *F. infectoria* inhibited protein denaturation by 67.07 and 47.17 percent, respectively, at a conc. of 500 g/mL, while indomethacin inhibited 92.36 percent at the same concentration. Tables 7 and Figure 6.

CONCLUSION

In comparison to benchmarks like ascorbic acid, rutin, and Indomethacin, the findings show that both alcoholic and aqueous preparations of *Ficus infectoria* fruits have high antioxidant and anti-

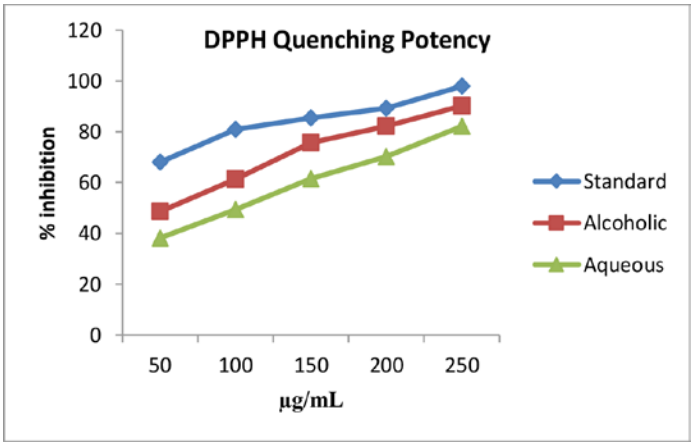

Figure 1: DPPH quenching potency of aqueous and alcoholic extract of *Ficus infectoria*.

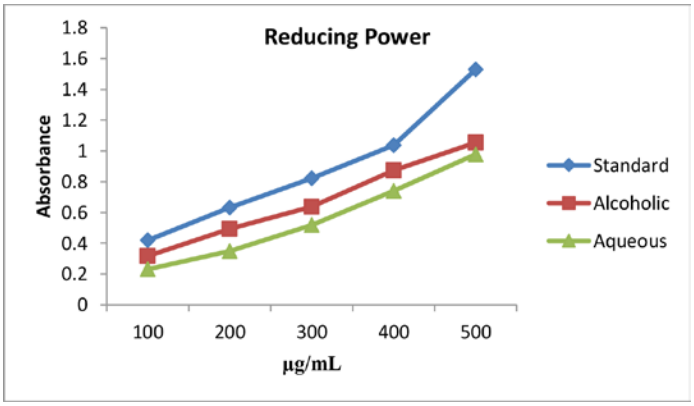

Figure 2: Reducing power potency of aqueous and alcoholic extract of *F. infectoria*.

Table 2: DPPH quenching potency of *Ficus infectoria*.

	% Inhibition of DPPH free radical of extracts		
Conc. (µg/mL)	Standard	Alcoholic	Aqueous
50	68.05 ± 0.81	48.58 ± 0.71	38.21 ± 0.15
100	80.89 ± 0.16	61.34 ± 1.03	49.34 ± 0.50
150	85.49 ± 1.05	75.71 ± 1.09	61.53 ± 0.47
200	89.18 ± 0.57	82.12 ±0.22	70.19 ± 0.53
250	97.89 ± 0.16	90.17 ± 0.30	82.12 ± 0.33

Table 3: Reducing power potency of *Ficus infectoria*.

Conc. (µg/mL)	Standard	Alcoholic	Aqueous
100	0.419 ± 0.15	0.317 ± 0.10	0.231 ± 0.30
200	0.632 ± 0.36	0.494 ± 0.31	0.349 ± 0.10
300	0.821 ± 0.80	0.637 ± 0.26	0.519 ± 0.34
400	1.036 ± 0.47	0.874 ± 0.41	0.742 ± 0.38
500	1.529 ± 0.42	1.054 ± 0.33	0.978 ± 0.51

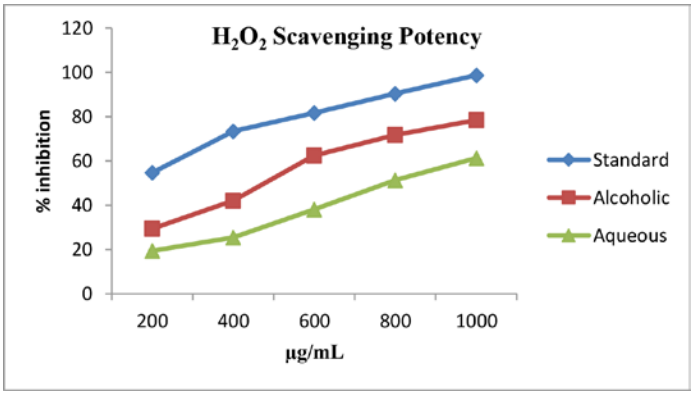

Figure 3: H_2O_2 scavenging potency of alcoholic and aqueous extract of *F. Infectoria*.

Table 4: Hydrogen peroxide scavenging potency of *Ficus infectoria*.

	% Inhibition of H_2O_2		
Conc. (µg/mL)	Standard	Alcoholic	Aqueous
200	54.67 ± 0.18	29.37 ± 0.36	19.37 ± 0.37
400	73.43 ± 0.37	42.12 ± 0.23	25.45 ± 0.21
600	81.77 ± 0.27	62.39 ± 0.52	38.04 ± 0.14
800	90.27 ± 1.06	71.72 ± 0.18	51.38 ± 0.33
1000	98.72 ± 0.70	78.41 ± 0.23	61.25 ± 0.23

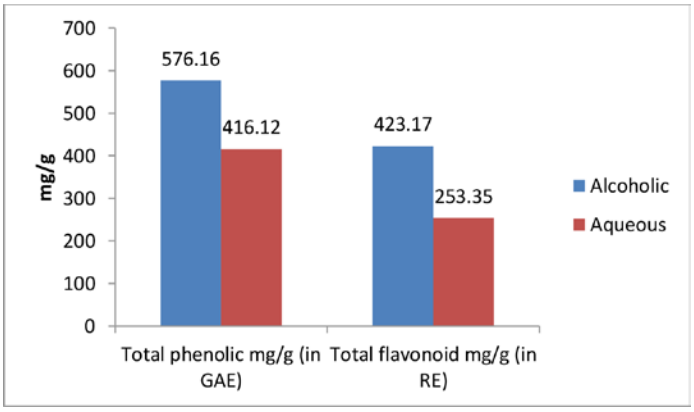

Figure 4: Total Phenolic and flavonoid content of *Ficus infectoria* fruits.

Table 5: Total phenolics and flavonoid content of *F. infectoria* (Mean ± S.E.M.).

Extract	Total phenolic mg/g (in GAE)	Total flavonoid mg/g (in RE)
Alcoholic	576.16 ± 129.10	423.17 ± 56.48
Aqueous	416.12 ± 112.01	253.35 ± 24.07

(a): average of three determinations

Table 6: % Membrane Stabilization potency of *Ficus infectoria*.

Conc. (µg/mL)	Standard	Alcoholic	Aqueous
25	52.62 ± 0.43	32.31 ± 0.38	26.93 ± 0.28
50	65.14 ± 1.03	41.69 ± 0.32	32.16 ± 0.22
75	77.51 ± 0.47	53.83 ± 0.43	43.57 ± 0.15
100	89.81 ± 0.10	68.76 ± 0.28	58.18 ± 0.13
125	96.76 ± 0.30	82.09 ± 1.20	65.47 ± 1.70

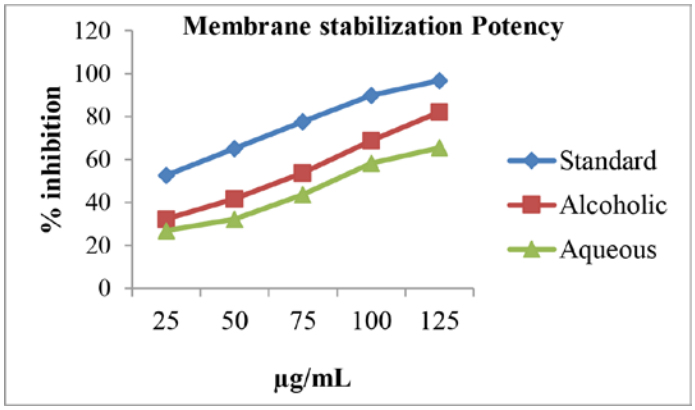

Figure 5: *In vitro* membrane stabilization potency of methanolic and aqueous extracts of *F. infectoria*.

Table 7: Protein denaturation potency of *Ficus infectoria*.

Conc. (µg/mL)	Standard	Alcoholic	Aqueous
100	45.18 ± 0.90	22.10 ± 0.18	12.24 ± 0.19
200	62. 34 ± 0.09	31.64 ± 0.48	19.56 ± 0.28
300	74. 02 ± 0.32	39.73 ± 0.53	26.86 ± 0.06
400	85.10 ± 0.07	53.12 ± 0.93	39.55 ± 0.24
500	92.36 ± 0.31	67.07 ± 0.83	47.17 ± 0.42

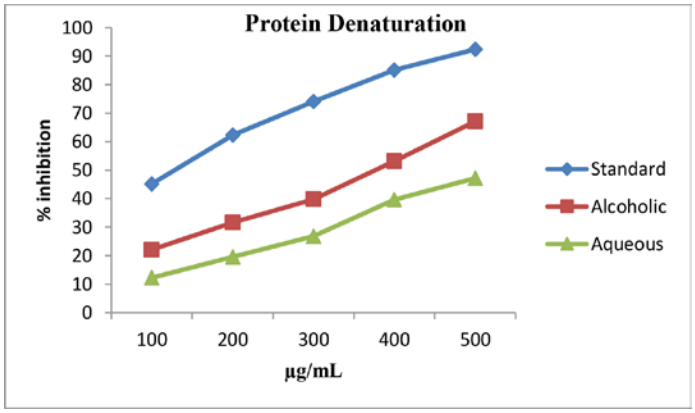

Figure 6: Protein denaturation potency of *Ficus infectoria*.

inflammatory activity. However, in comparison to aqueous preparation, alcoholic preparation demonstrated statistically prominent potency. To back up the current findings, more research is being done to isolate, identify, and describe the active principle(s).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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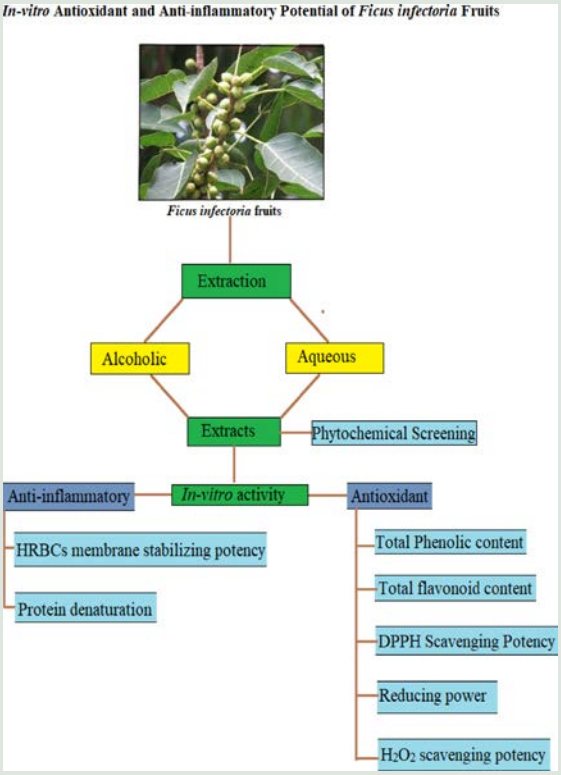
GRAPHICAL ABSTRACT



SUMMARY

Antioxidants are chemical substances, either synthetic or natural, that can avert or slow down cellular damage. Furthermore at the site of inflammation, free radicals involve other cellular components leads to loss of function and even death. *In vitro* research on the antioxidant properties of various natural and commercial foods, their components, and the methodologies created for these investigations have been ongoing for many years. Many fruits and veggies include flavonoids, phenolic acids, and carotenoids, according to research. Since a diet high in antioxidants is good to human health, research on organic items' antioxidant potential is conducted. Using *in vitro* techniques, the antioxidant and anti-inflammatory efficacy of *Ficus infectoria* fruits was assessed. Compared to ascorbic acid, both alcoholic and aqueous extracts quenched DPPH and H_2O_2. HRBC membrane stabilizing and protein denaturation model was used to assess anti-inflammatory potency. Compared to indomethacin, both extracts were anti-inflammatory. Compared to ascorbic acid, both formulations had high reducing power. Both formulations have excellent antioxidant and anti-inflammatory action.

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