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## Protective effects of aqueous and ethanol extracts of *Ficus religiosa* Leaves and Bark on H<sub>2</sub>O<sub>2</sub>-induced oxidative DNA damage in human lymphocytes by comet assay

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### Abstract

*Ficus religiosa* is traditionally used as medicine to treat various diseases which include Otitis media, Mastitis, Diabetis, Pharyngolaryngitis, Dysmenorrhea and Urethritis. The present study deals with the protective effects of the aqueous and the ethanol extracts of *Ficus religiosa* leaves and bark on H<sub>2</sub>O<sub>2</sub> – induced oxidative DNA damage in human lymphocytes by the comet assay.

Fresh leaves and bark of *Ficus religiosa* were collected from Draksharam, Bhimeswara swamy temple to perform antioxidation and comet assay. The leaves and bark were washed under running tap water, air dried and powdered finely and used for further studies.

The results showed that the tail lengths are increased when the samples are treated with the hydrogen peroxide. Increased tail length denotes that the more damage is occurred to the genetic material and the damage is increasing along with the increase in the concentration of hydrogen peroxide.

Among the four extracts of *Ficus religiosa* (Leaf Ethanolic extract, Leaf aqueous extract, Bark Ethanolic extract, bark aqueous extract) leaf Ethanolic extract has more antigenotoxic activity because, the inhibition percentage of the Leaf Ethanolic extract and the p value of this is statistically very significant. The nature of antigenotoxicity is increasing with the increasing concentration. Even at the low concentration of this extract, significant inhibition rate is observed.

**Keywords:** *Ficus religiosa*, antioxidation, comet assay, genotoxicity and antigenotoxicity

### Introduction

There is the availability of high biodiversity on the countries which are located on the tropical and the sub tropical regions. Knowledge of use of plants for the purpose of disease treatment has been passed from generation to generation. *Ficus Linn* is the largest genus of the family Moraceae comprising of about 755 fig tree species worldwide (Van NS *et al.*, 2007) <sup>[1]</sup>. *Ficus religiosa* commonly known as Bodhi tree is one of the most important species of this genus. Among these plants, *Ficus family* is one, it is a well known ethnomedicinal tree used in ayurveda. It is used in the preparation of traditional medicine for more than 50 types of disorders, almost every part of this plant is used for treatment of various diseases. Leaf juice along with honey is used for treatment of asthma, cough, sexual disorders, diarrhoea, haematuria, earache and toothache, migraine, eye troubles, gastric problems (Kattel LP and Kurmi PP, 2004) <sup>[2]</sup> and scabies. Fruits are used for the treatment of asthma and respiratory disorders. Fruit paste is taken to cure scabies. Stem bark is used in the treatment of gonorrhoea, bleeding, cuts, wounds, paralysis, diabetes, diarrhea, bone fracture and used as antiseptic, astringent and antidote. Bark paste along with honey is used to treat cough and cold as well as accompanying mild fever. Aerial root juice is used for treatment of menstrual problems (Ripu MK and Bussmann WR, 2006) <sup>[3]</sup>. Charaka and sushrutha prescribed a decoction of the bark of ashvattha in haemorrhages; leaves for covering wounds; the paste of the tender roots or the bark for skin infections. Sushrutha administered a decoction in urinary disorders and vaginal discharges.

Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. But there is a need to replenish these antioxidant compounds because these are used up in the process of neutralizing free radicals.

Herbal antioxidants have been successfully employed as rejuvenators, for several centuries in the Indian systems of alternative medicine (Blokina O *et al.*, 2003; Rice EC *et al.*, 1997; Van NS *et al.*, 2007) [1, 4, 5]. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

Genetic Toxicology is the modern branch of toxicology. It deals with the study and characterization of chemical and physical agents that damage the hereditary material of living organisms (Albart P. and Li RH, 1991) [6]. Some toxicants damage the genetic complexes at concentrations also producing acute non-specific cytotoxicity and death. The primary objective of genetic toxicology is to detect and analyze the hazard potential of those agents that are highly specific for interactions with nucleic acids and produce alterations in genetic elements at subtoxic concentration (Brusic D, 1987) [7]. Components at subtoxic exposure levels, resulting in modified hereditary characters or DNA inactivation, are classified as genotoxic (Ehrenberg LB, 1973) [8].

Among the known techniques in genetic toxicology, the comet assay is one of them. The comet assay is also called as single-cell gel electrophoresis. It was first developed by Ostling & Johansson in 1984 and later changed by Singh *et al.* in 1988. It is a noninvasive technique compared with the other techniques (chromosomal aberrations, micronucleus) which require larger samples (2-3ml) as well as proliferating cell population (or cell culture). The assay has been widely used in studying DNA damage and repair in healthy individuals, in clinical studies as well as in dietary intervention studies and in monitoring the risk of DNA damage resulting from occupational exposures, environmental, oxidative damage or life style. The term "comet" refers to the pattern of deoxyribonucleic acid migration through the cataphoresis gel, which frequently resembles a extraterrestrial body. At molecular level, the formation of "comets" in the DNA of cells upon the genotoxic insult can be visualized through the method of gel electrophoresis and indicates DNA strand breaks, as the damaged DNA migrates at a different rate than non damaged DNA during electrophoresis. In the comet assay, when the damaged DNA-containing single cell suspension embedded in low melting agarose is electrophoresed, the damaged DNA migrates away from the undamaged DNA-containing nucleoid body, resembling the structure of a comet. In the comet structure, the undamaged DNA nucleoid part is referred to the "head" and the trailing damaged DNA streak is referred to as "tail".

Nowadays all over the world looking for traditional medicine, it is revalued by wide-ranging of research on different plant species and their therapeutic principles. The objective of the study was to evaluate the antigenotoxicity and antioxidant properties of *Ficus religiosa* bark and leaf.

### Material and methods

Fresh leaves and bark of *Ficus religiosa* were collected from, Bhimeswara swamy temple in Draksharam, East Godavari district of the Indian state of Andhra Pradesh. The leaves and bark were washed under running tap water, remove debris and then rinsed with distilled water several times. The leaves were allowed to shade dry for 3-5 days. The dried samples were blended into fine powder and stored in containers. About 10 gms of sample powders (in the ratio

of 1:10 (powder/solvent)) were taken in clean sterile Soxhlet apparatus and extracted with 100 ml of water and methanol. After extraction the extracts were dried in hot air oven. From the solid extract was made into suitable concentrations using DMSO for further analysis.

### Isolation of human lymphocytes

Obtain human blood from a healthy donor. Gently pipette 3 mL of room temperature ficoll into an 8 mL round-bottom polystyrene tube. Gently add 3 mL of whole blood on top of the ficoll. It is necessary to avoid combining of the two reagents. Centrifuge the tubes at 500 x g for 45 minutes at room temperature. Following the centrifugation, the peripheral blood mononuclear cells (PBMC) have now separated from other blood components into the top cell layer. The PBMC layer seems, from the highest down, because the initial cloudy band. Carefully remove the clear yellow-colored upper phase of the blood, above the PBMC layer, and then use a P1000 micropipette to transfer the PBMC layer to a 15 mL or 50 mL conical tube. Wash the PBMC twice with PBS, centrifuging cells at 500 x g for 5 minutes each time. The supernatant will be somewhat cloudy after each wash (Criag T *et al.*, 2010) [9].

### Total phenolic content using Folin-Ciocalteu phenolic assay (TPC)

#### Preparation of calibration curve using gallic acid as standard

10mg of standard gallic acid was weighed and dissolved in 100mL of dis.H<sub>2</sub>O in the volumetric flask (100µg/mL of the stock solution). Pipette out aliquots of 0.5 to the 2.5mL into 25mL of the volumetric flasks from the above stock solution. 10mL of dis.H<sub>2</sub>O was added and 1.5 mL of the Folin Ciocalteu reagent was diluted according to the label which are specified on each of the above volumetric flasks. Add 4mL of 20% of sodium carbonate solution after 5 minutes and make up the volume upto 25mL with dis.H<sub>2</sub>O. The absorbance was recorded at 765 nm after 30 minutes and the calibration curve of absorbance vs concentration was plotted.

#### Preparation of test solution

Accurately weighed 1g of powdered leaves and bark was extracted with (3 × 15mL) 50% ethanol and aqueous extract by cold soaking for two hours with the alternating shaking. Later it is filtered and final volume of the ethanolic extract and aqueous extract of both the leaves and bark was made separately up to 50mL each. 1mL was pipette out from this test solution into 25mL volumetric flask and the same procedure was performed for color development using the Folin-Ciocalteu reagent. The total amount of phenolics of gallic acid was calculated using standard curve (Permender Rathee *et al.*, 2010; Singleton VL *et al.*, 1965) [10,11].

### Free radical scavenging activity (FRSA) using hydrogen peroxide

The hydrogen peroxide FRSA of the methanolic extracts was done as suggested by Czochra and Widensk. (Czochra MP and Widensk AJ, 2002) [12] 2ml of hydrogen peroxide (43 m mol) and 1.0 ml of methanolic sample [20-100 µl of methanolic extract (4 mg/ml) of plant in methanol] followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4) were added. The resulting solution was kept for 10 min and the absorbance was recorded at 230 nm. All readings were

repeated three times. Blank was prepared without adding hydrogen peroxide and control was prepared without sample. Ascorbic acid was used as a standard compound. Free radical scavenging activity of hydrogen peroxide (%) was calculated as  $FRSA (\%) = [(V_0 - V_1)/V_0] \times 100$ , where,  $V_0$  = absorbance of control and  $V_1$  = absorbance of sample.

### Comet assay

The fresh blood was taken and the lymphocytes are extracted. The extracted lymphocytes are used as samples for testing.

The Ficus extracts are first tested for genotoxicity and later for antigenotoxicity. The genotoxic nature is tested by treating the sample with the extracts at different concentrations (150 $\mu$ l, 200 $\mu$ l, 250 $\mu$ l, 300 $\mu$ l) and then kept for half an hour. After that comet assay is done using this samples. The antigenotoxic nature is tested by treating the sample with H<sub>2</sub>O<sub>2</sub> and then with the extracts at different concentrations immediately. Then kept for half an hour and it is used as sample for comet assay.

In this experiment four different extracts of *Ficus religiosa* are tested for their antigenotoxic activity i.e., Leaf Ethanolic Extract, Leaf Aqueous Extract, Bark Ethanolic Extract and Bark Aqueous extract.

\* Note: F.L.Et = *Ficus* Leaf Ethanol Extract.

F.L.Aq = *Ficus* Leaf Aqueous Extract.

F.B.Et = *Ficus* Bark Ethanol Extract.

F.B.Aq = *Ficus* Bark Aqueous Extract.

Dust free, plain slides were covered a layer of 140  $\mu$ l of 0.67% NMA and allowed to dry for 10mins in hot air oven. About 110  $\mu$ l of NMA was layered as second layer and was immediately covered with cover slip and was kept at 4 $^{\circ}$  c for 10mins. 20  $\mu$ l of blood sample (Approximately 1000-5000 cells) was mixed with 110  $\mu$ l of warm LMA and mixture was layered as third additional layer and gelled at 4 $^{\circ}$  c for 10mins. A fourth additional layer of 110  $\mu$ l of LMA was added on top and gelled again in the similar way as mentioned above. After fourth layer of gel was set, the slides were treated overnight in freshly prepared chilled lysing buffer solution at 4 $^{\circ}$  c with this treatment the cell membrane and nuclear membrane were lysed and the majorities of proteins were removed to expose the nucleosides. The slides were then removed from the lysing solution, drained and placed in a horizontal electrophoresis tank side by side avoiding spaces and with agarose end facing the anode. The tank was filled carefully with fresh electrophoresis buffer to a level approximately 0.25cms above the slides. The slides were left in the high PH (PH>13) buffer for 20mins to allow unwinding DNA. Electrophoresis was carried out at room temperature for 40mins at 3000mA, 20 V. After electrophoresis, the slides were flooded 3 times gently with chilled neutralizing solution (Tris PH 7.5) for 5mins (Rahman Gul SU, 2017; Debiyi OO and Sofowora FA, 1978; Roopashree TS *et al.*, 2008; Sofowora A, 1993; Trease GE and Evans WC, 1989; Nandhan Kumar S, 2011; Ostling O and Johanson KJ, 1984; Singh NP *et al.*, 1988; Collins AR, 2003; Collins AR *et al.*, 1993; Dusinska M and Collins AR, 1996)<sup>[13- 23]</sup>. The slides were silver stained by the method of Ahiya and Saran 1999. Briefly air dried slides were immersed in the fixing solution for 10mins and washed gently with distilled water several times. The washed slides were allowed to air dry for about 1hour before staining. 68ml of ss (B) was mixed with 32ml

of ss (A) and poured over the dried slides so as to cover the slide uniformly. This step was repeated with a fresh mix of ss until a grayish/brakish silver color developed on the slides. Hundred consecutive cells (50 cells from each end of slide) were manually selected and quantified with, which also determined the olive tail moment parameter (McGlynn AP *et al.*, 1999; Gedik CM *et al.*, 1992; Santos SJ *et al.*, 1997; Parasuraman S *et al.*, 2010; Nadin SB *et al.*, 2001; Somorovska M *et al.*, 1999; Jenkinson AM *et al.*, 1999; PoolZobel BL *et al.*, 1997; Jayaprakash T *et al.*, 2010; Collins A *et al.*, 1997)<sup>[24- 33]</sup>.

### Statistics

The percentage of inhibition is calculated to the samples treated with different extracts of different concentration using the following formula:

% of Inhibition = [(normal activity - inhibited activity) / (normal activity)] \* 100%

Inhibition (%) = a - b/a - c  $\times$  100

Where a = Tail length induced by H<sub>2</sub>O<sub>2</sub> (positive control).

b = Tail length of the compound in the presence of H<sub>2</sub>O<sub>2</sub>.

c = Tail length of the negative control

### Results and discussion

Fresh leaves and bark of *Ficus religiosa* were collected from draksharamam and are washed thoroughly under tap water after air dry we made fine powder. The aqueous and ethanolic extracts were prepared from this powders and used to measure antioxidant genotoxicity and antigenotoxicity activities.

Phenolic compounds are the class of antioxidant agents that act as free radical terminators (Shahidi F and Wanasundara PKJPD, 1992) <sup>[34]</sup>. Among the plant constituents, Phenolic compounds are very important because they act as scavengers due to the presence of hydroxyl group; they also act as powerful chain breaking antioxidants and have been associated with antioxidant activity (Sharma SK and Gupta VK, 2008) <sup>[35]</sup>. The total Phenolic Content in ethanol and aqueous extracts of *Ficus religiosa* leaf and bark were determined by using the Folin-Ciocalteu method and were expressed as Gallic Acid Equivalent per gram dry weight of plant extract.

The maximum phenolic content was found in ficus bark ethanolic extract (120 $\pm$ 1.5 mg GAE/ gm powder weight) while the lowest phenolic content was found in ficus leaf aqueous extract (96 $\pm$ 0.96 mg GAE/ gm powder weight) (Graph 1). The total phenolic content of the ficus leaves and bark ethanolic and aqueous extracts are in the following decreasing order like bark ethanolic extract > leaf ethanolic extract > bark aqueous extract > leaf aqueous extract. The total results of the ficus leaves and bark extracts of ethanolic and aqueous are shown in the table 1.

Results related to research work of Mun Hui Sin, Awang Soh Mamat *et al.*, <sup>[36]</sup> have sorted the present research outcomes. They evaluated the total phenolic content potential of *Ficus deltoidea* using green and non-green solvents. Among the solvent extracts, aqueous was the most extractible solvent of phenols, that varied from 368.42 $\pm$  6.37 mgGAE/g, followed by ethanolic extract that varied from 263.45 $\pm$  5.28 mg/g and our results varied like, we got more in ethanol extract than the aqueous solvent which varied from 120 $\pm$ 1.5 mgGAE/g of bark and 108 $\pm$ 0.64 of leaf followed by aqueous extract that varied from 104 $\pm$ 0.84 of bark and 96 $\pm$ 0.96 of leaf.

Hydrogen peroxide can directly inactivate few enzymes usually of the essential thiol (-SH) group by oxidation and it is a weak oxidizing agent. Hydrogen peroxide once inside the cell can rapidly cross the cell membrane, and can probably react with  $\text{Cu}^{2+}$  ions and  $\text{Fe}^{2+}$  to form the hydroxyl radical which may be the origin of many of its toxic effects (Kumar Hemant *et al.*, 2011) [37].

The maximum result was found in *F. religiosa* bark ethanolic extract with 106% where as the minimum was found in *F. religiosa* leaf aqueous extract with 99% (Graph 2). The total result of free radical scavenging by  $\text{H}_2\text{O}_2$  of *F. religiosa* leaf and bark ethanol and aqueous extracts are in the following decreasing order like bark ethanolic extract > leaf ethanolic extract > bark aqueous extract > leaf aqueous extract. The total results of the ficus leaves and bark extracts of ethanolic and aqueous are shown in the table 1.

The Comet assay is a standard method for measuring DNA damage. In this study, the protective effects of aqueous and ethanol extracts of *Ficus religiosa* on human lymphocyte DNA lesions were evaluated with the comet assay. Lymphocytes were isolated from the fresh blood taken from the healthy volunteers. The lymphocytes are treated

preliminarily with  $\text{H}_2\text{O}_2$  to check DNA damage (Table 2). The genotoxicity of the ficus extracts are tested through treating the lymphocytes with extracts in different concentrations (150,200,250,300 $\mu\text{g/ml}$ ) (Table 3). Then the antigenotoxicity of extracts are tested by treating lymphocytes initially with  $\text{H}_2\text{O}_2$  (20 $\mu\text{M}$ ) and then with the extracts in different concentrations (150,200,250,300 $\mu\text{g/ml}$ ) (Table 4). In the lymphocytes pretreated with the  $\text{H}_2\text{O}_2$  and extracts, there was reduction in the DNA damage compared to control (untreated) detected through comet assay.

The tail lengths are increased when the samples are treated with the hydrogen peroxide. Increased tail length denotes that the more damage is occurred to the genetic material and the damage is increasing along with the increase in the concentration of hydrogen peroxide. At the concentration of 5 $\mu\text{M}$  (Table 3) the increased tail length is statistically not significant. But when the concentration is increased to 30 $\mu\text{M}$  it becomes more significant. At the concentration of 20 $\mu\text{M}$ , there is 0.04 p value (Table 4). There is a considerable increase in the tail length. This concentration is further used for the induction of damage into the genome of cells.

**Table 1:** Total Phenolic Content (TPC) and Free Radical Scavenging Activity (FRSA) using Hydrogen Peroxide of *Ficus religiosa* Leaf and Bark Ethanol and Aqueous extracts

Plant extracts	(FRSA) using Hydrogen Peroxide %	TPC (mg of GAE/100 gm powder weight)
F.L.Aq	68%	96 $\pm$ 0.96
F.L.Et	99%	108 $\pm$ 0.64
F.B.Aq	89%	104 $\pm$ 0.84
F.B.Et	106%	120 $\pm$ 1.5

**Table 2:** Effect of Hydrogen Peroxide on human peripheral blood lymphocytes

Hydrogen peroxide	Cells scored	P value	Tali length $\pm$ SE
Untreated	100	NS	0.4 $1 \pm$ 0.12
5 $\mu\text{M}$	100	0.09	0.85 $\pm$ 0.16
10 $\mu\text{M}$	100	0.04	1.46 $\pm$ 0.33
15 $\mu\text{M}$	100	0.03	2.56 $\pm$ 0.67
20 $\mu\text{M}$	100	0.04	3.15 $\pm$ 0.91
30 $\mu\text{M}$	100	0.01	4.05 $\pm$ 0.94

NS-Non Significant

**Table 3:** Non-mutagenic effects of selected compounds by using DNA damage

Compounds	Cells scored	Tail length $\pm$ SE	P value
Untreated	100	0.4 $1 \pm$ 0.12	NS
DMSO	100	0.61 $\pm$ 0.14	0.3391
L.Et (150 $\mu\text{g/ml}$ )	100	0.98 $\pm$ 0.34	0.1896
L.Et (200 $\mu\text{g/ml}$ )	100	0.84 $\pm$ 0.28	0.2309
L.Et (250 $\mu\text{g/ml}$ )	100	0.82 $\pm$ 0.29	0.2615
L.Et (300 $\mu\text{g/ml}$ )	100	0.71 $\pm$ 0.26	0.3539
L.Aq (150 $\mu\text{g/ml}$ )	100	0.71 $\pm$ 0.27	0.3674
L.Aq (200 $\mu\text{g/ml}$ )	100	1.05 $\pm$ 0.31	0.1265
L.Aq (250 $\mu\text{g/ml}$ )	100	1.25 $\pm$ 0.29	0.05
L.Aq(300 $\mu\text{g/ml}$ )	100	1.35 $\pm$ 0.39	0.08
B.Et(150 $\mu\text{g/ml}$ )	100	0.49 $\pm$ 0.18	0.73
B.Et (200 $\mu\text{g/ml}$ )	100	0.61 $\pm$ 0.24	0.4975
B.Et (250 $\mu\text{g/ml}$ )	100	0.75 $\pm$ 0.28	0.3269
B.Et (300 $\mu\text{g/ml}$ )	100	0.91 $\pm$ 0.34	0.2378
B.Aq (150 $\mu\text{g/ml}$ )	100	0.88 $\pm$ 0.28	0.1977
B.Aq (200 $\mu\text{g/ml}$ )	100	0.99 $\pm$ 0.33	0.1739
B.Aq (250 $\mu\text{g/ml}$ )	100	1.10 $\pm$ 0.38	0.1584
B.Aq (300 $\mu\text{g/ml}$ )	100	1.14 $\pm$ 0.41	0.1627

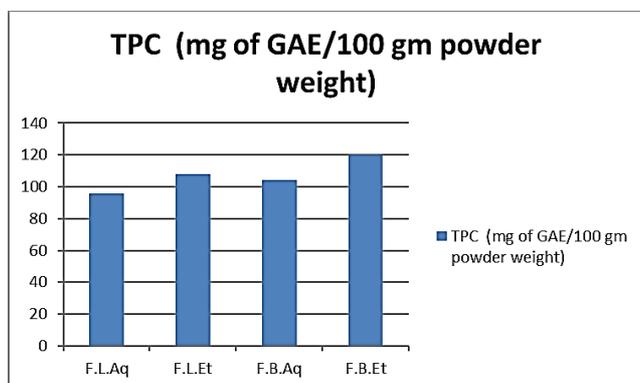
\*NS-Non significant

**Table 4:** Antigenotoxic effect of following extracts against H<sub>2</sub>O<sub>2</sub> Induced DNA damage

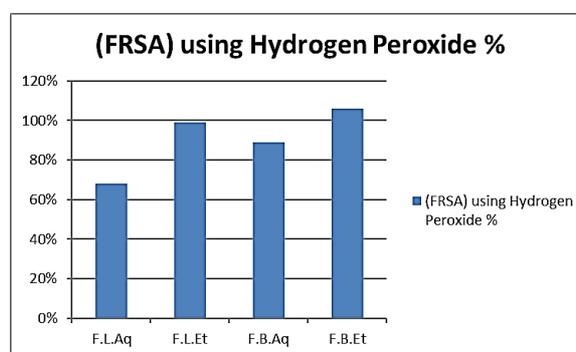
Hydrogen peroxide (20 µM) + Extract (µg/ml)	Cells scored	Tail length ±SE	P value	% of inhibition
Untreated	100	0.4 1± 0.12	NS	NS
20+0(Positive control)	100	3.15 ±0.91	0.04	NS
L.Et 20+150	100	2.29+/-0.56	0.03	33.8
20+200	100	1.91+/-0.39	0.02	48.8
20+250	100	1.34+/-0.31	0.04	71.2
20+300	100	0.96+/-0.31	0.17	86.2
L.Aq20+150	100	3.00 +/-0.59	0.006	27.7
20+200	100	2.95+/-0.54	0.01	39.3
20+250	100	2.09+/-0.39	0.014	41.7
20+300	100	1.94+/-0.35	0.014	47.6
B.Et 20+150	100	1.99+/-0.38	0.016	45.6
20+200	100	1.45+/-0.24	0.017	66.9
20+250	100	1.04+/-0.16	0.03	83
20+300	100	0.98+/-0.19	0.06	85.4
B.Aq 20+150	100	2.04+/-0.33	0.002	43.7
20+200	100	1.87+/-0.38	0.02	50.3
20+250	100	1.81+/-0.26	0.008	52.7
20+300	100	1.51+/-0.41	0.06	64.5

\*NS = Non significant

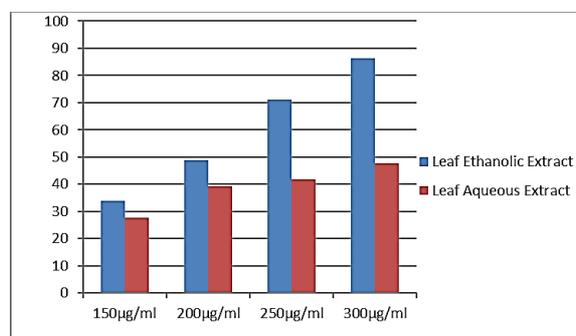
Among the four extracts of *Ficus religiosa* (Leaf Ethanolic extract, Leaf aqueous extract, Bark Ethanolic extract, bark aqueous extract) leaf Ethanolic extract has more antigenotoxic activity because, the inhibition percentage of the Leaf Ethanolic extract at 300µg/ml concentration is 86.2% and the p value of this is 0.17 (Graph 3) which is statistically very significant. The nature of antigenotoxicity is increasing with the increasing concentration. Even at the low concentration (150µg/ml) of this extract, significant inhibition rate is observed (33.8%). After the leaf Ethanolic extract, Bark ethanolic extract has more antigenotoxic activity. The percentage of inhibition at 150µg/ml concentration of this is 45.6% (Graph 4) which is statistically significant. It shows its antigenotoxic nature even present at low concentrations. At high concentrations (300µg/ml) the inhibition percentage is 85.4 and it is statistically significant. The Bark aqueous extract also has significant antigenotoxic property. This nature increases with the increase in concentration. The inhibition percentage of this extract at 300µg/ml concentration 64.5% (Graph 4) and is statistically significant. The Leaf aqueous extract has low antigenotoxic property among these four extracts. The inhibition percentage at high concentration (300µg/ml) is 47.6% (Graph 3) which is nearer to the inhibition percentage of Bark Ethanolic extract at 150µg/ml concentration. But the value is statistically significant.



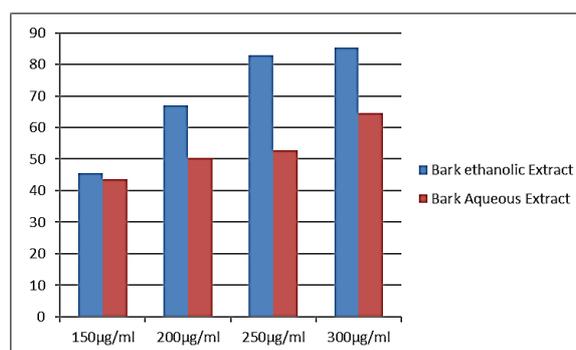
**Graph 1:** Total Phenolic Content (TPC) of *Ficus religiosa* Leaf and Bark Ethanol and Aqueous extracts



**Graph 2:** Antioxidative test against TBA of *Ficus religiosa* Leaf and Bark Ethanol and Aqueous extracts



**Graph 3:** Inhibition percentages of Leaf ethanolic and aqueous extracts at different concentrations.



**Graph 4:** Inhibition Percentages of Bark ethanolic and aqueous extracts at different concentrations.

The samples treated with only extracts in different concentrations shows somewhat increase in the tail lengths. But this increase is not considerable because the p values are not significant. By this experiment it is known that the *Ficus religiosa* extracts does not have genotoxic nature.

Results related to research work of Mohammed, *et al.* have sorted the present research outcomes. They evaluated that *A.Cavens* and *A.Furcatispina* showed low or insignificant genotoxic nature at (0.1 and 10 mg/mL) and at 20 mg/mL showed significant genotoxicity (Ana Mariel Mohamed *et al.*, 2016) [38] and our results are highly significant compared to their. Among the solvent extracts, we got the low genotoxicity of *Ficus religiosa* at 5 $\mu$ M and a significant genotoxicity at 30 $\mu$ M concentration. More over *Ficus religiosa* showed a significant antigenotoxicity also.

### Conclusion

The present study is the comparative evaluation of Total Phenolic Content, antioxidant and antigenotoxicity activity of the ethanolic and aqueous extracts of leaves and bark of *Ficus religiosa* were performed. From our results, it was concluded that the ethanolic extracts of bark and leaves of *Ficus religiosa* contain the significant amount of phenolic, antioxidating and genotoxic contents. These results demonstrate that *F. religiosa* aqueous and ethanol extracts of leaves and bark are not genotoxic and are antigenotoxic to the H<sub>2</sub>O<sub>2</sub> induced DNA damage in lymphocytes. Among these four extracts, the ethanol extract of bark has high antigenotoxic property. The Ethanolic Extracts of leaf and bark shows more antigenotoxic nature when compared to the aqueous extracts. So the use of these extracts in the drug preparation of traditional medicine is more useful when compared to the aqueous extracts of leaf and bark.

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