

# Phytochemical Profiling and Anti-Microbial Activity of *Ficus Religiosa* Against *E. Coli* and *S. Aureus*

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## Abstract

Plants have been used for thousands of years to keep people well and enhance the quality of their lives by acting as useful components in medicines, food, beverages, cosmetics, and dyes. *Ficus religiosa* (Sacred fig) or Peepal tree is known to be a native Indian tree. Extract of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-convulsant activity, anthelmintic activity, proteolytic activity, anti-immunomodulatory activity, wound-healing activity, antioxidant activity, anti-acetylcholinesterase activity, wound healing activities and antidiabetic activity. The aqueous extract of *F. religiosa* dried bark shown the presence of flavonoids, tannins, phytosterols and bergapten. The stem bark of *F. religiosa* investigation shows phytochemical component of tannins, phenols, flavonoids, alkaloids and steroids, vitamin K, n-octacosanol, methyl oleanolate, lanosterol,  $\beta$ -sitosterol-D-glucoside, stigmasterol, lupen-3-one. Four different extracts (Aqueous, Hydro-alcohol, Methanol, Acidified Water) of leaf of *F. religiosa* were taken for the experiment, from which it concludes that Alkaloid, Tannin, and Resin are present in all the extracts. Whereas, Flavonoid, and Cardiac glycoside are absent in all. The result of Thin Layer Chromatography shows highest Rf value of 0.90 in Hydro-alcohol extract and lowest in Methanol (Rf value of 0.55). The anti-microbial activity of Acidified water extract shows the highest zone of inhibition of 15 mm and 18 mm; however, aqueous extract gives the lowest zone of inhibition of 10 mm and 12 mm against *E. coli* and *S. aureus*, respectively. *F. religiosa* exhibit many pharmacological activities i.e., anticonvulsant, antidiabetic, analgesic, wound-healing, antioxidant, acetylcholinesterase, proteolytic, anti-amnesic and many more. These discovered chemicals are what give *Ficus religiosa* its significant biological functions. In order to find new medicines, the researcher will be instructed to find and extract the novel therapeutic compounds from different parts of *Ficus religiosa*.

**Keywords:** *Ficus religiosa*, Rf value, anti-inflammatory, phytochemical screening, crude extracts, anti-microbial activity, methanol, hydro-alcohol, acidified water, distilled water.

## Introduction:

Since ancient times, people have been exploring nature especially plants, in search of new drugs, and

this has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases. In India, almost 95% of the prescriptions have been reported to be plant based in the traditional systems of AYUSH, which is an acronym for Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy [1].

*Ficus religiosa* (Sacred fig) or Peepal tree is known to be a native Indian tree. It is a large evergreen tree found throughout India. It is a familiar sight in Hindu temples, Buddhist monasteries, villages and at roadsides. It is widely being used to treat various ailments like skin diseases, vomiting, burns, nervous disorder, heart diseases, diabetes, constipation, dysentery, snakebite. Extract of the plant possess significant anti-diarrhoeal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-convulsant activity, anthelmintic activity, proteolytic activity, anti-immunomodulatory activity, wound-healing activity, antioxidant activity, anti-acetylcholinesterase activity, wound healing activities and antidiabetic activity [2].

Taxonomical classification of *Ficus religiosa*: Domain: Eukaryota; Kingdom: Plantae; Subkingdom: Viridiaeplantae; Phylum: Tracheophyta; Subphylum: Euphyllophytina; Infraphylum: Radiatopses; Class: Magnoliopsida; Subclass: Dilleniidae; Superorder: Urticales; Order: Urticales; Family: Moraceae; Tribe: Ficeae; Genus: *Ficus*; Species: *Ficus religiosa* L [3].

Traditionally the bark is used as an antibacterial, antiprotozoal, antiviral, astringent, antidiarrhoeal, in the treatment of gonorrhoea, ulcers and the leaves used for skin diseases. The leaves reported antivenom activity and regulates the menstrual cycle [4].

When the leaves first appear, their colour is red-pinkish, but then they turn deep green and grow to about 12-18 cm long. The leaves have 6-8 pairs of side-veins and a further network of very fine veins. This delicate venation and the ability of the leaf to disintegrate easily in water are both clearly illustrated in the greeting cards which are sometimes made with peepal leaves [5].

Phytochemical screening is a critical step in the isolation of new and innovative chemicals. A crucial step before bulk extraction and isolation is phytochemical screening. *Ficus religiosa* fruits possess an adequate quantity of flavonoids named as kaempferol, quercetin, and myricetin and phenolic compounds. Asparagine and tyrosine are the amino acids which are found in fruit pulp of *Ficus religiosa*. The seeds of this tree indicate the presence of phytosterolin,  $\beta$ -sitosterol, and its glycoside, albuminoids, carbohydrate, fatty acids, colour imparting agent, caoutchou 0.7–5.1% [6]. The aqueous extract of *F. religiosa* dried bark shown the presence of flavonoids, tannins, phytosterols and bergapten. The stem bark of *F. religiosa* investigation shows phytochemical component of tannins, phenols, flavonoids, alkaloids and steroids, vitamin K, n-octacosanol, methyl oleanolate, lanosterol,  $\beta$ -sitosteryl-D-glucoside, stigmaterol, lupen-3-one [7]. Approximately 4.9% of the fruit is having protein with vital amino acids, phenylalanine and isoleucine. The phytochemical analysis of the leaves reported the presence of phytosterols, triterpene alcohols, long-chain hydrocarbons, aliphatic alcohols, amino acids, tannins and minerals etc [8].

## Materials and Methods:

### Collection Of Samples:

Leaves of *Ficus religiosa* was collected from the local market of Bhubaneswar, Odisha and rinsed with distilled water and shade dried in the laboratory of SBio Science Pvt Ltd.

**Preparation Of Extracts:**

Dried leaves were grinded into fine particles with the help of mixer, and then stored into air tight container. Four separate beakers were taken and 30 ml of distilled water, hydro alcohol, methanol, and acidified water were added in these separate containers to get four different solvent extracts. 3 g of samples were immersed in each solvent and incubated at room temperature for 24 hours. The samples were then filtered into separate beakers with the help of filter paper [9].

**Phytochemical Screening:**

Quantitative assay for presence of secondary metabolites was performed using Standardized methods for the phytochemical analysis of the plant extracts.

**Detection of Alkaloids:**

Taken 1ml of plant extract and added 3-5 drops of Wagner's reagent and observed for the formation of reddish-brown precipitate or colouration, if positive.

**Detection of Carbohydrates:**

Taking 1ml of plant extract and adding 3-5 drops of Molisch's reagent, along with this 1ml of concentrated sulphuric acid ( $H_2SO_4$ ) was added to the side of the test tube. Then allowed the mixture to stand for 2-3 mins. Then observed the formation of red or dull violet colour at the interface of the two layers is a positive result.

**Detection of Cardiac Glycosides:**

Taken 1ml of extract and treated with 1ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution. To that mixture added 0.5 ml of conc.  $H_2SO_4$ . Observed for a brown ring or violet ring at the interface which shows the presence of glycosides.

**Detection of Flavonoids:**

1ml extract was treated with 3-5 drops of a 20% NaOH solution. The presence of flavonoids is indicated by the production of a bright yellow hue that becomes colourless after the addition of 0.5 ml dilute HCl.

**Detection of Phenols:**

Taking 1ml of extract and adding 5-6 drops of 5% aqueous ferric chloride solution and observed for the formation of deep blue or black colour.

**Detection of Proteins:**

Taken 1 ml of extract and added 2-5 drops of Ninhydrin solution and kept it in a boiling water bath for 1-2 min and observed for the formation of purple colour.

**Detection of Saponins:**

Take 1 ml of extract and added 5 ml of Distilled water and shake vigorously. Observed for the formation of persistent foam for 10-15 min that confirms the presence of saponins.

**Detection of Tannins:**

1 ml of the extract was treated with 1 ml of 10% alcoholic ferric chloride solution and the production of blue or greenish hue was noticed.

**Detection of Terpenoids:**

Take 1 ml of extract and added 0.5 ml of chloroform along with 3-5 drops of conc.  $H_2SO_4$ . Observed for the reddish-brown precipitate produced immediately.

**Detection of Resins:**

Take 1 ml of extract and added 5 ml of distilled water and observed for turbidity.

**Detection of Coumarins:**

Taking 1 ml of extract and adding 1.5 ml of 10% NaOH then observed the formation of yellow colour which indicates the presence of coumarins.

**Thin Layer Chromatography:**

Each of the plant extracts were checked by Thin Layer Chromatography (TLC) method on TLC plate over silical gel. For each extract, the solvent system Benzene: Chloroform: Acetone = 3:1:1 was used as mobile phase. In each case, the spots were visualized by naked eye and UV transilluminator [10].

**Antimicrobial Activity Test:**

The antimicrobial activity was determined by agar well diffusion method. 100 µl of *E. coli* and *S. aureus* cultures were spread in agar plate. Four wells were punched and 50 µl of different sample extracts were loaded and was allowed to diffuse further it was kept in incubator at 37°C for 18 hours. Next day zone of inhibitions were measured. The antibacterial activity test was based on the measurement of the diameter of the zone of inhibition formed around the well [11].

**Results and Discussion:**

**Phytochemical Screening:**

Leaves of *Ficus religiosa* was extracted by using four different solvents viz., Hydro-Alcohol (HA), Acidified Water (AW), Methanol (M), and Distilled Water (DW) by keeping them for up to 24 hours at room temperature.

Preliminary studies for the presence and absence of thirteen bio-molecules were carried out by different qualitative screening procedures which is shown in table.1.

Phytochemicals	<i>Ficus religiosa</i> Sample Extracts			
	Hydro-Alcohol	Acidified Water	Methanol	Distilled Water
Alkaloid	+ve	+ve	+ve	+ve
Flavonoid	-ve	-ve	-ve	-ve
Carbohydrate	+ve	+ve	+ve	-ve
Protein	+ve	-ve	+ve	+ve
Cardiac glycoside	-ve	-ve	-ve	-ve
Phenols	-ve	-ve	-ve	+ve
Steroids	+ve	-ve	-ve	-ve
Tannin	+ve	+ve	+ve	+ve
Terpenoid	+ve	-ve	-ve	-ve
Saponin	+ve	+ve	-ve	+ve
Resin	+ve	+ve	+ve	+ve
Coumarin	-ve	+ve	+ve	-ve
Quinone	+ve	-ve	-ve	+ve

[Table.1: Qualitative Screening of metabolites of different extracts of *Ficus religiosa*]

**Thin Layer Chromatography:**

The TLC was carried out to observe separated molecules. Mobile phase was prepared with Benzene: Chloroform: Acetone in the ratio of 3: 1: 1, respectively. The *R<sub>f</sub>* values were then measured and presented in the table.2.

Sample Extracts ( <i>Ficus religiosa</i> )	Spot Observed	<i>R<sub>f</sub></i> value
Hydro Alcohol (HA)	Brown	0.90
	Brown	0.61
	Purple	0.58
Acidified Water (AW)	Light Brown	0.86
	Brown	0.63
Methanol (M)	Green	0.66
	Off White	0.55
Distilled Water (DW)	Brown	0.77
	Brown	0.55

[Table.2: Retardation factor for different extracts of *Ficus religiosa*]

**Antimicrobial Assay:**

The plant extracts were then subjected to antimicrobial screening. The result of Acidified water extract shows the highest zone of inhibition; however, aqueous extract gives the lowest zone of inhibition against *E. coli* and *S. aureus*.

Anti-Microbial Activity Against	Zone of Inhibition (mm) of <i>Ficus religiosa</i> extracts			
	Hydro Alcohol (HA)	Acidified Water (AW)	Methanol (M)	Distilled Water (DW)
<i>E. coli</i>	13.0	15.0	0	10.0
<i>S. aureus</i>	0	18.0	14.0	12.0

[Table.3: Zone of inhibition (in mm) of *Ficus religiosa* against *E. coli* and *S. aureus* bacteria]

The fruit of *F. religiosa* contained total phenolic contents, total flavonoid, and percent inhibition of linoleic acid [12]. The Methanol extract of bark showed the presence of flavonoids, saponins, steroids, wax, terpenoids, cardiac glycosides and tannin [13,14]. Also, the bark extracts contain bergapten, bergapton, lanosterol, stigmasterol, lupen-3-one, vitamin k1 [15,16,17].

Hydro-alcohol (70%) extracts shows complete inhibition of the growth of *Helicobacter pylori* at 500 µg/ml in all strains and demonstrate anti-*H. pylori* activity with MBC value that ranged from 125 to 250 µg/ml [18]. Chloroform extracts showed an inhibitory activity against the growth of *S. typhi*, *S. typhimurium* and *P. vulgaris* at a MIC of 39, 5 and 20 µg/ml, respectively [19].

The Aqueous extracts showed decreased in blood glucose in fasting condition and increased in body weight at 100 and 200 mg/kg dose in diabetic rats as compared to untreated rats [20]. The methanolic extract of stem bark was administered to check the anti-inflammatory action in Wistar albino rats and also for detecting the analgesic action in Swiss albino mice [21].

**Conclusion:**

The world is blessed with an abundant supply of medicinal plants. Rural areas of developing nations with insufficient health facilities, depend heavily on medicinal plants for a variety of purposes. The results of this experiment are to find the bioactive compounds present in the four different extracts of *F. religiosa* and antimicrobial activity against *E. coli* and *S. aureus*. Four different extracts (Aqueous, Hydro-alcohol, Methanol, Acidified Water) of leaf of *F. religiosa* were taken for the experiment, from which it concludes that Alkaloid, Tannin, and Resin are present in all the extracts. Whereas, Flavonoid, and Cardiac glycoside are absent in all. The result of Thin Layer Chromatography shows highest Rf value of 0.90 in Hydro-alcohol extract and lowest in Methanol (Rf value of 0.55).

The anti-microbial activity of Acidified water extract shows the highest zone of inhibition of 15 mm and 18 mm; however, aqueous extract gives the lowest zone of inhibition of 10 mm and 12 mm against *E. coli* and *S. aureus*, respectively. This research provides empirical and scientific justification for the traditional anti-inflammatory, anti-diabetic properties of this plant. The findings of this study might also be useful commercially to pharmaceutical companies and research institutes for developing new medicines.

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**Conflict of Interest:**

Nil

**References**

1. Jiwala SA, Bagul MS, Parabia M and Rajani M. Evaluation of free radical scavenging activity of an Ayurvedic formulation. *Indian Journal of Pharmaceutical Sciences* 2008; 70:31-35.
2. Tripathi R, Kumar A, Kumar S, Prakash S and Singh AK (2015). *Ficus benghalensis* Linn.: A tribal medicine with vast commercial potential. *Indian J. Agric. Allied Sci.*, 1(3): 95-102.
3. Sharma Deepika, Dr. Dangi C.B.S., Kaur Manpreet. "A Review on Pharmacological Activities and Therapeutic Potentials of *Ficus Religiosa* (Pipal)". *Indian Journal of Applied Research*, 2016; 6(1): 623-626.
4. Kaur Amandeep, Rana A. C., Tiwari Vineeta, Sharma Ramica and Kumar Sunil. "Review on Ethanomedicinal and Pharmacological Properties of *Ficus religiosa*". *Journal of Applied Pharmaceutical Science*, 2011; 1(8): 06-11.
5. Kumari Kamini, Dr. Prajaptai Virendra, Ms. Jangir Surbhi, Mr. Ramratan. "A Review on *Ficus Religiosa* Moraceae: Distribution, Traditional Uses and Pharmacological Properties". *Journal of Biomedical and Pharmaceutical Research*, 2022; 11(5): 45-54.
6. Collins MA, Charles HP (1987). Antimicrobial activity of Carnosol and Ursolic acid: two anti-oxidant constituents of *Rosmarinus officinalis* L. *Food Microbiol.* 4:311-315.
7. Valsaraj R, Pushpangadan P, Smitt UW, Adersen A, Nyman U (1997). Antimicrobial screening of selected medicinal plants from India. *J. Ethnopharmacol.* 58:75-83.
8. Preeti R, Devanathan V, Loganathan M (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food Borne pathogens. *Advances Biol. Res.* 4:122-125.

9. Tanya, M., Priyadarsini, D., & Nayak, R. K. (2022). Screening of Bioactive Compounds from Madagascar Periwinkle and Their Importance in Drug Discovery. *IJFMR-International Journal for Multidisciplinary Research*, 4(6).
10. Tanya, M., Mishra, S. P., Das, A. P., Acharya, B., Priyadarsini, D., & Nayak, R. K. Physicochemical and Antioxidant Evaluation of Moringa Oleifera Leaf Extract Followed by Phytochemical Screening and Antibacterial Activity. *IJFMR-International Journal For Multidisciplinary Research*, 5(1).
11. Das, A. P., Priyadarsini, D., & Nayak, R. K. Extraction of Fruit & Seed from Black Grape & Fenugreek using Different Solvents for Phytochemical Screening followed by Anti-Microbial Activity Against E. Coli.
12. Swami, K. D., & Bisht, N. P. S. (1996). Constituents of *Ficus religiosa* and *Ficus infectoria* and their biological activity. *Journal of the Indian Chemical Society*, 73(11).
13. Babu K, Shankar SG, Rai S (2010). Comparative pharmacognostic studies on the barks of four *Ficus* species. *Turk. J. Bot.* 34:215–224.
14. Uma B, Prabaker K, Rajendran S (2009). In vitro antimicrobial activity and phytochemical analysis of *Ficus religiosa* and *Ficus benghalensis* L., against enterotoxigenic *E. coli*. *Food Chemical toxicology* (11)2842–6.
15. Joseph B, Justin SR (2010). Phytopharmacological and Phytochemical Properties of Three *Ficus* Species - An Overview. *Int. J. Pharm. Biol. Sci.* 1(4).
16. Margareth BCG, Miranda JS (2009). Biological Activity of Lupeol. *International journal of biomedical and pharmaceutical sciences* 3 (Special Issue 1):46–66.
17. Swami KD, Malik GS, Bisht NPS (1989). Chemical investigation of stem bark of *Ficus religiosa* and *Prosopis spicigera*. *J. Indian Chem. Soc.* 66:288–289.
18. Zaidi SFH, Yamadab K, Kadowakia M, Usmanghanic K and Sugiyamab T (2009). Bactericidal activity of medicinal plants, employed for the treatment of gastrointestinal ailments, against *Helicobacter pylori*. *J. Ethnopharmacol.* 121:286–291.
19. Hemaiswarya S, Poonkothai M, Raja R, Anbazhagan C (2009). Comparative study on the antimicrobial activities of three Indian medicinal plants. *Egypt J. Biol.* 1:52–57.
20. Kottapalli Kameswara Jayant, Buddhan Sundaram Vijayakumar. “In-vitro anti-oxidant and anti-diabetic potential of endophytic fungi associated with *Ficus religiosa*”. *Italian Journal of Mycology*, 2021;50:10-20.
21. Sreelekshmi R, Latha P G, Arafat Mm, Shyamal S, Shine V J, Anuja G I, Suja S R And Rajasekharan S. “Anti-Inflammatory, Analgesic and Anti-Lipid Peroxidation Studies on Stem Bark of *Ficus Religiosa* Linn”. *Natural Product Radiance*, 2007; 6(5):377-381.