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Research Article

Phytochemical Screening Photoluminance Study and TLC (Thin Layer Chromatography) of *Ficus Religiosa* Leaves Extract

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ABSTRACT

Ficus religiosa is a member of the family Moraceae. It is common in southern India. After phytochemical screening, the aqueous extract of *Ficus Religiosa*'s bark, leaves, stem, and fruits showed the presence of alkaloids, phenol, sugar, terpenoids, glycosides, flavonoids, and tannins. *Ficus religiosa* leaves are picked for phytochemical analysis. The extracts contained terpenoids, flavonoids, and tannins, among other types of useful secondary metabolites. The various parts of this plants like stem bark, fruits, buds, are used in treatment of different diseases like dysentery, mumps, jaundice, heart diseases, constipation, skin diseases, etc. *Ficus religiosa* is useful in diabetes. *F. religiosa* showed a wide range of pharmacological properties, including wound-healing, antioxidant, acetylcholinesterase, anti-amnesic, anticonvulsant, antidiabetic, anti-inflammatory, and antibacterial properties. Preliminary phytochemical screening of *F. religiosa* barks, showed the presence of tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides. Leaves yield campesterol, stigmasterol, isofucosterol, α -amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tyrosine, methionine, valine, isoleucine, leucine

INTRODUCTION

The foundation of our traditional medical system is made up primarily of different bioactive compounds, which are primarily found in medicinal plants. In the field of medicine, phytoconstituents obtained from medicinal plants

are important. Many kinds of plants have been used in traditional medicine to cure various kinds of diseases. In the fields of medicine, many plants and herbs are highly valued, but *Ficus religiosa*, a member of the Moraceae family, is one of the most crucial. In Sanskrit, it can be referred to as "sacred

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fig," "Peepal," or "Ashwattha." Since it is one of the sacred trees worshipped by Hindus, it may be found all over India.[1] The bark, fruits, leaves, seeds, and resin of the *F. religiosa* species tree are the main natural remedies used to treat a variety of illnesses. The primary cause of plants' beneficial benefits is their secondary metabolites, which have qualities that improve health. A variety of active phytoconstituents including flavonoid, polyphenolics, tannins, terpenoids, saponins, plant steroids, glycosides etc. [2]*Ficus religiosa* is a member of the family Moraceae. It originated in south-east Asia, ranging from India. Bark powder is used as hypoglycaemic medication. Stem bark has spasmolytic properties on smooth muscles and is used to treat skin conditions, ulcers, asthma, parasympatholytic infections, viral infections, bacterial infections, and protozoan infections.[3] Toothache can be relieved by latex. Fruit extract is utilized as a purgative, and has anticancer potential. Medicinal plants are increasingly included into food supplements, nutraceuticals, cosmetics, and medications. Medicinal plants contain some natural products which perform physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. The leaf juice is traditionally used to treat gastrointestinal problems, asthma, diarrhoea, coughing, migraines, eye, ear, and toothaches. Toothaches is treated with the decoction of leaves. The fruit is used to cure respiratory issues such as asthma and scabies. The stem bark was used as an antiseptic and astringent, and to treat bone fractures, gonorrhoea, paralysis, diabetes, and diarrhoea.[4]

Common name of *Ficus religiosa* (peepal Tree)

F. religiosa, a member of the Moraceae family, is more widely referred to as the peepal tree. Bodhi tree, peepul tree, and ashwattha tree are some more names for it. Asthma, diabetes, diarrhoea, epilepsy, stomach issues, inflammatory disorders,

viral diseases, and sexual disorders can all be effectively treated with this medicinal plant. The leaves are reddish-pink when they first arrive, but as they grow to be between 12 and 18 cm long, they turn a dark shade of green. The leaves involve an additional network of extremely small veins in addition to 6–8 pairs of side veins. The various parts of this plants like stem bark, fruits, buds, are used in treatment of different diseases like dysentery, mumps, jaundice, heart diseases, constipation, skin diseases, etc. *Ficus religiosa* is useful in diabetes. *F. religiosa* showed a wide range of pharmacological properties, including wound-healing, antioxidant, acetylcholinesterase, anti-amnesic, anticonvulsant, antidiabetic, anti-inflammatory, and antibacterial properties. In traditional medicine, *Ficus religiosa* has been widely used to treat a variety of conditions affecting the central nervous system, endocrine system, gastrointestinal tract, reproductive system, respiratory system, and infectious disorders.[5]

Plant profile:-

Domain: Eukaryota

Kingdom: Plantae

Subkingdom: Viridiaeplantae

Order: Urticales

Phylum: Tracheophyte

Family: Moraceae

Genus: *Ficus*

Species: *F. religiosa*

Ecology

F. religiosa flowers in February and fruits in May to June. New leaves appear in April. It is mostly found in India. It is extensively distributed in uplands and level areas, but it is also found rarely in forests, where it grows as an epiphyte on other trees.[6]

Plant Morphology

Large deciduous tree *F. religiosa* has few or no aerial roots, with its drooping branches bearing long, petiole, ovate, cordate, glossy leaves, it is frequently epiphytic. The apex of the bright green



leaves forms a linear-lanceolate tail that is roughly half the length of the main leaf. The pairs of receptacles are axillary, depressed globose which on ripening becomes smooth and purplish. The bark is between 5 and 8 mm in size, either flat or slightly curved, thickness, has a grey or ash exterior with thin, membrane-like flakes, and is frequently covered in brown or ash-coloured crustose lichen with shallow, erratic vertical fissures and uneven because of the cork's exfoliation, the inner surface is smooth, fibrous, and yellowish to orange brown. [7], [8]

Phytogeography:

Although it depends on the plantation, it has an oriental distribution on a global scale, meaning it is widely distributed throughout India. In the

Traditional uses:

Indian subcontinent, it can be seen thoroughly, or to state that it is indigenous to India lower continent. It is distributed in Sub-Himalayan regions of India: Bengal Forest, Maharashtra, Haryana, Punjab, Gujarat, and Uttar Pradesh, Rajasthan and Madhya Pradesh.[9], [10]

Phytochemistry:

Preliminary phytochemical screening of *F. religiosa* barks, showed the presence tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides. Leaves yield campesterol, stigmasterol, isofucosterol, α -amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine.[11]

Table1: - The main uses of plant parts of *Ficus religiosa*

Sr no.	Plant part and its uses
1.	Bark: Astringent, cooling, aphrodisiac, antibacterial against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i> , gonorrhea, diarrhea, dysentery, hemorrhoid's and, anti-inflammatory, burns
2.	Leaves and tender shoots: Purgative, wounds, skin diseases
3.	Leaf juice: Asthma, cough, sexual disorders, diarrhea, hematuria, toothache, migraine, eye troubles, gastric problems, scabies

Identical names: -

Sanskrit: Pippala

Assamese: Ahant

Bengali: Asvattha, Ashud, Ashvattha

English: Pipal tree

Guajarati: Piplo, Jari, Piparo, Pipalo

Hindi: Pipala, Pipal

Kannada: Arlo, Ranji, Basri, Ashvatthanara, Ashwattha, Ashvathamara, Basari,

Kashmiri: Bad

Malayalam: Arrayal

Marathi: Pipal, Papal, Pippal

Oriya: Aswath

Punjabi: Pipal, Pippal

Tamil: Ashwarthan, Arasamaram, Arasan, Arasu, Arara

Telugu: Ravichettu

Pharmacological activities:-

It shows various pharmacological activity. A broad range of in vitro and in vivo pharmacological activities, including antidiabetic, anti-inflammatory, wound healing, anticonvulsant, anti-inflammatory, analgesic, antimicrobial, antioxidant, antiasthmatic, antitumor, antiulcer, antianxiety, and proteolytic activity, were demonstrated by fresh plant materials, crude extracts, and extracted components of *F. religiosa*. [12]

1. Antibacterial activity

Aqueous and ethanolic extracts of *F. religiosa* leaves showed antibacterial effect against *Staphylococcus aureus*, *Shigella dysenteriae*, *S.*

typhimurium, Pseudomonas aeruginosa, Bacillus subtilis, S. aureus, Escherichia coli in another study, chloroform extract of fruits showed antimicrobial effect against Azobacter chroococcum, Bacillus cereus, B. megaterium, Streptococcus faecalis, Streptomycin lactis, and Klebsiella pneumonia. The ethanolic extract of leaves showed antifungal effect against Candida albicans. Aqueous, methanol, and chloroform extracts from the leaves of F. religiosa were completely screened for antibacterial and antifungal activities[13], [14]

2. Antimicrobial activity

Microbes are microorganisms, including bacteria, viruses, fungi, and others, that may cause infectious and deadly diseases if acquired into any biological system. An antimicrobial agent refers to natural or synthetic components that can kill or inhibit the growth of those microorganisms.[15], [16]

3. Anti-inflammatory activity

The methanolic extract of stem bark of F. religiosa has shown significant anti-inflammatory activities orally. A significant anti-inflammatory effect has been observed in acute and chronic models of inflammation; the extract also protected mast cells from degranulation induced by various degranulate s.

4. Anti-ulcer activity

The disease known as a "peptic ulcer" is characterized by a discontinuity in the thickness of the duodenum or stomach mucosa, which continues due to the presence of pepsin and acid in the stomach juice. According to Rang et al. (5th edition), the main pathological conditions for which it is beneficial to lower acid secretion are reflux a condition known as peptic ulceration (both duodenal and stomach). [17]The use of commercially available antiulcer medicines for treating peptic ulcers is typically accompanied by a number of negative effects. Finding new antiulcerogenic compound(s) with maybe fewer

or no side effects is therefore necessary. Ficus religiosa is one of the plants that has been traditionally used in folk medicine in India as well as Malaysia to treat stomach ulcers.[18], [19]

5. Anti-asthmatic activity

Bronchial asthma is another condition that is treated with Ficus religiosa. The antiasthmatic properties of Ficus religiosa ethanol leaves extract was initially studied by Malhotra et al. The extract had an inhibitory effect on experimental asthma produced by histamine and acetylcholine.[10]

MATERIAL AND METHODS

Collection of plant:- Ficus religiosa leaves were collected from local area Mandi (Himachal Pradesh) in February (2024) and removed foreign particles Wash the leaves with water to remove dirt and debris and dried at 30-40°C in an oven.

Authentication:-

The plant is identified and authenticated by Dr. Pankaj Sharma (H.P. State Biodiversity Board) Shimla (Himachal Pradesh).

Letter no.- HPSBB/277.

MACROSCOPIC STUDY

Morphological characteristics:

Morphological characteristics of Ficus religiosa such as plant structure, leaves, flowers, fruit, texture, colour and peeling sections were studied by visual observations and verified with standard taxonomical books.

Organoleptic study:

Organoleptic properties such as odour, taste and touch were visually and sensory observed. The information that was correctly collected, recorded and documented.

Extraction of Plant material:

Preparation of extract: -Ficus religiosa leaves were collected from Sarkaghat Mandi (Himachal Pradesh) in February (2024) and removed foreign particles Wash the leaves with water to remove dirt and debris and dried at 30-40°C in an oven. The plant is identified and authenticated by Dr. Pankaj



Sharma (H.P. State Biodiversity Board) Shimla (Himachal Pradesh).

Grinding: - Grind the dried leaves into a fine powder using a mortar pestle or a grinder and stored the leaves powder into tight packet.

Methanolic extraction of plant leaves

Soxhlet Extraction: -

10gm of leaves powdered is weighed properly and dissolved into 100ml of methanol. Set up Soxhlet apparatus. place the plant material into the thimble of the Soxhlet extractor. Put 100ml of methanol into the round-bottom flask of the Soxhlet apparatus. The extracting solvent in the flask is heated, causing its vapours to condense in the condenser and extraction procedure was performed for three days, approximately 50 cycles and then the samples were then filtered into separate beakers with the help of Whatman filter paper. The solvents were removed under reduced pressure on rotary evaporator to get dry extracts at 50°C with 180 rpm. The extracts were then allowed to cool and stored for further use.

PHOTOLUMINESCENCE STUDIES:

In photoluminescence spectroscopy, or PL for short, light energy, or photons, are used to induce a photon to be emitted from any material. It is a non-destructive, non-contact technique for probing materials. Basically, light passes onto a sample, where it is absorbed and can trigger a process known as photo-excitation. The material gets excited by photons, which cause it to jump to a higher electronic state. As it relaxes and returns to a lower energy level, photons are released. Photoluminescence, or PL, is the process by which light or luminescence is emitted.

Impurity levels and defect detection: -

At low sample temperatures, the photoluminescence spectroscopy spectrum frequently displays spectral peaks linked to impurities present in the host material. This technique's high sensitivity makes it possible to detect incredibly low concentrations of both

intentional and unintentional impurities, which can have a significant impact on the quality of the material.

Recombination mechanisms: -

The relative amounts of radiative and nonradiative recombination rates are directly correlated with the amount of photoluminescence spectroscopy that a material emits. Since nonradiative rates are usually linked to impurities, this method can qualitatively track how growth and processing conditions affect the quality of the material. [20]

PHYTOCHEMICAL SCREENING

1. Test for Glycosides

Legals test

After taking 1 ml of the extract, 1ml of sodium nitroprusside, a small amount of sodium hydroxide solution, and shaking were added. A pink to blood red precipitate's formation indicates the presence of a glycoside.[21]

2. Test for Carbohydrate

Molisch's test

Take 1ml of extract is taken and add 2ml of Molisch's reagent. Now to this mixture 2ml conc. Sulfuric acid was added along the side of test tube. Presence of carbohydrates was confirmed by formation of reddish violet ring at the junction of two liquids.

3. Flavonoid test

Lead acetate test

Take 1ml of the extract will be taken and put into a test tube in order to look for flavonoids. After that, shake well and add a few drops of lead acetate. When a yellow precipitate forms, flavonoids are present.[14]

Alkaline reagent test

Take 1ml plant extract is taken, 1ml of aqueous NAOH was added. Presence of flavonoids is confirmed by the formation of yellowish colour.

4. Test for Saponins

Take 1ml extract is taken and 5ml water was added to it, shaken well in test tube shaker.



Presence of saponin is confirmed by lather formation.

5. Test for Tannins

Ferric chloride test

Take 1ml plant extract is taken and 1ml of ferric chloride was added to it. Presence of tannins is confirmed by the formation of greenish black colour.

Gelatine Test:

A test tube will be filled with 1ml of extract. Next, add the sodium chloride containing 1% Gelatine solution and shake. The presence of tannins is indicated by the appearance of white precipitate.

Borntrangers Test:

Add 0.1 g of the drug, 5 ml of dilute HCl, and 5 ml of 5% solution of ferric chloride. Boil for a few minutes. Cool and filter. Shake the filtered part with benzene. The separated benzene layer and equal volume of dilute solution of ammonia shows pink colour.[22]

6. Test for alkaloids

Dragendroff's reagent:- Take 1ml of plant extract add 2-3 drops of Dragendroff's reagent were added. Presence of alkaloid is confirmed by formation of creamish, brownish red, orange precipitate.

Mayers Test: -

Take 1 ml of extract and transfer to a test tube. Then add 1ml of Mayers Reagent (potassium mercuric iodide solution) and shake well. The appearance of white, creamy, precipitate indicates the presence of alkaloids.

Wagner's test:-

A test tube will be filled with 1 ml of extract. Wagner's reagent, 1 mL of potassium iodide, will be then added and shaken. A reddish-brown precipitate's appearance indicates the presence of alkaloids.[23]

7. Detection of phenols

Take 1ml of extract and treated with 2-3 drops of ferric chloride solution. Formation of bluish black colour confirmed the presence of phenols.

THIN LAYER CHROMATOGRAPHY (TLC)

Preparation of Stationary Phase: - The sample was applied to TLC-Precoated Plates that had silica gel G F254 (a fluorescent indicator) on them, 1cm above the baseline.

Development of Chromatographic Chamber: -

A glass container with a water and methanol (1:1) solvent system was used to create the chromatographic chamber. To eliminate the edge effect, this chromatographic chamber was pre-saturated.

Development of Chromatogram: -

The stationary plate containing the sample was put into the pre-saturated chromatographic chamber so that the solvent or mobile phase was below the sample application spot. The sample was eluted through the TLC plate by the capillary action mechanism. [24]

Scanning and detection of the spots: -

The TLC plates were left to air dry. Using a UV chamber exposed to UV-visible radiation, including both near- and far-UV radiation, the spots were scanned and found. We computed the R_f value of spots using the following formula:

$$R_f = \frac{\text{Distance travelled by spot from origin}}{\text{Distance travelled by solvent front}}$$

RESULT AND DISCUSSION: -

MACROSCOPIC STUDY

1. Morphology

Size: -

Ficus religiosa is a large, deciduous tree that can reach heights of up to 30 meters (98 feet) tall.

Trunk: -

The trunk is straight and cylindrical, with a diameter of up to 3 meters (9.8 feet). It often has a smooth, light grey bark that becomes fissured and rough with age.

Branches: -

The tree has spreading branches that form a wide canopy, creating a dense shade. These branches

may have aerial roots that grows downward from the branches and eventually reach the ground, providing additional reach the ground, providing additional support to the tree.

2. Leaves

Shape: -

The shape of leaves is typically cordate or heart-shaped, with a long and a round base.

Arrangement: -

The leaves are arranged alternately on the branches.

3. Flowers

Structure: -

The flowers are tiny and enclosed within the syconium, with both male and female flowers present within the same structure.

Pollination: -

The flowers are pollinated by specific fig wasps that enter the syconium to lay their eggs.

4. Fruits

Type: -

The fruit of *Ficus religiosa* are technically syconia, which are fig-like structure. They are small, round, and green when immature, turning purple when ripe.

Seed: -

Each syconium contains numerous tiny seeds embedded within the fleshy interior.

5. Cultural significance

Holds immense cultural and religious significance in various cultures, particularly in Hinduism, Buddhism. It is often considered sacred and is associated with spiritual beliefs and rituals.

Macroscopic character of *Ficus religiosa* leaves:-

Morphological characters show colour, odour, size, shape and taste and other foliar morphological characters.

Table 2: Macroscopical characters of *Ficus religiosa* Leaves

Sr.no.	Morphological character	Observation
1.	Colour	Fully grown Leaf – Deep Green Soft Leaf – Rose to Light Red
2.	Oduor	odorless
3.	Taste	Bitter taste
4.	Shape	Oval shape
5.	Size	Length – 14 - 22cm Width – 7 – 18 cm
6.	Texture	Thin, smooth

PHOTOLUMINESCENCE STUDY:

Under visible and ultraviolet light rays, the fluorescence of a powdered plant material with

different chemical reagents and extracts was observed.

Table No. 3: Fluorescence characteristics of *Ficus Religiosa* leaves:

Sr. No.	Treatment	Visible light	UV Light 254nm (Shorter Wavelength)	UV Light 365nm (Long Wavelength)
1.	Leaves Powder	Dark green	Greenish	Dark brown
2.	Methanolic extract	Dark green	Reddish brown	Dark purple
3.	Leaves Powder + 1N H ₂ SO ₄	Light Yellow	Light green	Dark green
4.	Leaves Powder + 1N HCL	Light Yellow	Light green	Greenish
5.	Leaves Powder + 1N NaOH	Black	Blood red	Dark Purple



Phytochemical test of Ficus religiosa leaves extract

Table4: - Phytochemical tests of Ficus religiosa leaves extract

Tests	Procedure	Result
Glycosides test (Legal test)	1ml plant extract+ 1ml of sodium nitroprusside+ a small amount of sodium hydroxide solution	Blood red precipitate occurs show the presence of glycosides.
Carbohydrate test (Molisch's test)	1ml extract+ 2ml Molisch's reagent+2ml conc. sulphuric acid	Formation of reddish violet ring at the junction of two liquids
Flavonoid Test		
1. Alkaline reagent test.	1ml plant extract+1ml aqueous NAOH	Formation of yellowish Colour
2. Lead acetate test	1ml of plant extract+ add few drops of lead acetate	Formation of yellow precipitate.
Saponins	1ml extract+5ml water. Shake it properly.	Formation of foam for 10-15 min it shows the presence of saponins.
Tannins Test		
1.Ferric chloride test	1ml extract +1ml ferric chloride.	Formation of greenish black Colour
2.Gelatine test	Add 1ml of extract + the sodium chloride containing 1% Gelatin solution and shake.	Formation of white precipitate
3. Bontrager's Test	Add 0.1 g of the drug + 5 ml of dilute HCl + 5 ml of 5% solution of ferric chloride. Boil for a few minutes. Cool and filter. Shake the filtered part with benzene	The separated benzene layer and equal volume of dilute solution of ammonia shows pink Colour.
Alkaloids Test		
1.Dragendroff's reagent	1ml of plant extract + 2-3drops of Dragendroff's reagent	formation of ceramic/ brownish red/ orange precipitates.
2.Mayer's test	1ml of plant extract + add 1ml of Mayer's reagent	white, creamy, precipitate indicates the presence of alkaloids.
Phenols	1ml of extract+ 2-3 drops of ferric chloride solution	bluish black Colour is formed.

PHYTOCHEMICAL SCREENING:

Various phytochemical tests were performed and obtained results are as follow:

Table5: - Phytochemical screening of Aqueous extract and Methanolic extract of Ficus religiosa

Phytochemical	Aqueous extract	Methanolic extract
Glycosides	-ve	+ve
Carbohydrate	+ve	+ve
Flavonoid		
1. Alkaline reagent test.	+ve	+ve
2. Lead acetate test	-ve	+ve
Saponins	+ve	+ve
Tannins		
1.Ferric chloride test	+ve	+ve
2.Gelatine test	-ve	-ve
3. Bontrager's	-ve	+ve
Alkaloids Test		
1.Dragendroff's reagent	-ve	+ve
2.Mayer's test	+ve	+ve
Phenols	+ve	+ve

Thin Layer Chromatography:

The methanol and distilled water extract was subjected to qualitative chromatography using methanol: distilled water (1:1) (MeOH: DW) as

the mobile phase. Four spots in the chromatogram of the methanolic extract were detected under 254 nm Ultra-Violet radiation.

Table 6: Rf value calculation in thin layer chromatography of Ficus religiosa leaves under 254nm.

Extract	Mobile Phase	Total run(cm)	Number of spots	Distance of Solvent front from origin	Distance of spot from origin (Sample application site)	Rf value
Methanolic extract	MeOH + DW (1:1)	3	4	7	4.6	0.657
					5.0	0.714
					5.2	0.742
					6.2	0.885

CONCLUSION:-

In conclusion, the preliminary phytochemical screening, Photoluminance study, and TLC analysis of leaf extracts from Ficus religiosa reveal a rich diversity of bioactive compounds with potential medicinal and industrial applications. Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigation into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Various chemical

compounds have been found in the Ficus religiosa leaves extract under research, according to preliminary phytochemical tests. The literature has a clear understanding of the various phytoconstituents' activities in the pharmaceutical fields. Thus, the specific phytochemical information provides advantages to the pharmaceutical industry. Additionally, several methods can be used to identify these phytochemicals in order to examine their effects separately or in combination. Plant extract could

be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments. extracts of leaves of plants have revealed the presence of alkaloids, flavonoids, glycosides, phenols, saponins, and tannins. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles subsequently may lead to the drug discovery and development.

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