IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Antimicrobial activity of *Ficus religiosa* leaves extract used for Treatment of Urinary Tract Infection

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ABSTRACT:- Urinary tract infections (UTIs) have become a growing problem in recent years. Most of Gram negative (GN) bacteria that cause urinary tract infections (UTIs) are *Escherichia coli*. Other Gram positive (GP) bacteria that cause UTIs include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Acinetobacter baumannii*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Citrobacter Freunde*, *Proteus vulgaris*, and *Klebsiella oxytocin*. Four microorganisms representing Gram positive and Gram negative were used. Two-gram positive bacteria were *Staphylococcus aureus* and *Bacillus subtilis* and two-gram negative bacteria were *Escherichia coli* and *Salmonella typhi*. antimicrobial activity of plant extracts was monitored by the agar-well diffusion method. In recent times, medicinal plants have found great popularity to treating different kinds of infections including urinary tract infections. Preliminary phytochemical analysis shows the presence of alkaloids, tannins, saponins, flavonoids as the active bio ingredients in F. religiosa leaves extract.

Keywords: UTI, *Ficus religiosa*, antimicrobial activity, Phytochemical analysis, TLC(Thin layer chromatography) Zone of inhibition, MIC and MBC.

INTRODUCTION:- Many societies have been used medicinal plants for thousands of years. Nowadays, using medicinal plants to create a healthy bodily environment is becoming more and more popular. Since long ago, they have proven helpful in the treatment of many different kinds of diseases and conditions, such as urinary tract infections(UTI).(1) UTI caused by bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, and *Proteus mirabilis*. All over the world, millions of people are diagnosed with urinary tract infections (UTI) every year. Mostly female are more affected by urinary tract infection(UTI). (2) A lot of plants have been investigated to treat the UTI(3), and other types of infection caused due to the pathogenic organisms. Plants are the basis of various complex traditional systems of medicine, including Chinese, Unani, and Ayurvedic.(4) It is well known that the peepal tree, or *Ficus religiosa*, is a native Indian tree. It's a big, evergreen tree that grows all over India. It is a common sight in roadside locations, Buddhist monasteries, and Hindu temples. Many conditions, including skin conditions, vomiting, burns, heart problems, neurological disorders, diabetes, constipation, dysentery, and snakebite, are treated with it on a regular basis. Profound anti-diarrheal, anti-fungal, anti-plasmodial, anti-ulcerogenic, anti-convulsant, anthelmintic, proteolytic, anti-immunomodulatory, anti-oxidant, anti-acetylcholinesterase, wound-healing, and antidiabetic properties are found in the plant's extract.(5)

These medical systems provided some medications that are still in use today. More than 80% of people around the world, according to the World Health Organization (WHO), get their primary medical treatment from traditional medicine.(6)

Urinary Tract Infection(UTI):-

The kidneys, ureters, bladder, and urethra make up the urinary system, which filters blood by eliminating waste materials and extra water. when microorganisms enter the urinary tract via the urethra, they infect the bladder and may lead to chronic Kidney infection.(7) One important mechanism in the elimination of metabolic waste products from the bloodstream is the urinary system. Blood pressure and volume regulation, as well as the normalization of ion and solute concentrations in the blood, are among the system's other crucial tasks.(8) Urine is either sterile or contains very few pathogenic microorganisms in healthy individuals. One of the most prevalent infectious diseases in the world, urinary tract infections (UTIs) can be caused by infections in the urethra , bladder, or kidneys (pyelonephritis). UTIs are divided into two categories: simple (UTIs) and complex (UTIs).(9)

Main causes of urinary tract infection:-

The most common cause of UTIs is the bacterium Escherichia coli (E. coli), which is commonly found in the digestive system. However, other bacteria such as Klebsiella, Proteus, Enterococcus, and Staphylococcus saprophyticus can also cause UTIs.(10)

UTIs can occur when bacteria enter the urinary tract through the urethra and begin to multiply in the bladder. This can happen due to various reasons:

1. **Poor Hygiene:** Improper wiping (from back to front) after using the toilet can facilitate the spread of bacteria from the anal region to the urethra(11).
2. **Waiting too long to urinate:** Holding urine for extended periods can allow bacteria to multiply in the bladder.
3. **Certain Health Conditions:** Conditions such as diabetes or a weakened immune system can increase the risk of UTIs.
4. **Obstruction:** Any blockages in the urinary tract, such as kidney stones, can prevent the complete emptying of the bladder, increasing the risk of infection.(12,13)

SYMPTOMS

Burning sensation at the start of urination Symptoms of Cystitis often include

a) Fever
b) Lower abdominal pain
c) Urine colour look cloudy, or red
d) Burning sensation during the urination(14,15)

Medicinal plant:-(peepal) *Ficus religiosa*

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. (16) 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.(17)

Plant profile:-

- **Kingdom:** Plantae
- **Division:** Magnoliophyta
- **Class:** Magnoliopsida
- **Order:** Rosales
- **Family:** Moraceae
- **Genus:** Ficus
- **Species:** religiosa
- **Common name:** Peepal.(18)

Plant morphology:-

Large tree *F. religiosa* has few or no aerial roots, with its drooping branches bearing long, ovate, cordate, glossy leaves, it is frequently epiphytic. *Ficus religiosa* is mostly found in India.(19) The apex of the bright green leaves forms a linear-lanceolate tail that is roughly half the length of the main leaf. 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.(20)

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 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.(21)

Phytochemistry:

Preliminary phytochemical screening of F. religiosa barks, showed the presence of tannins, saponins, flavonoids, steroids, terpenoids and cardiac glycosides. (22)Leaves yield campestrol, stigmasterol, isofucosterol, α-amyrin, lupeol, tannic acid, arginine, serine, aspartic acid, glycine, threonine, alanine, proline, tryptophan, tryosine, methionine, valine, isoleucine, leucine.(23)

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(24)

Traditional uses:

Sr no.	Plant part and its uses
1.	**Bark:** Astringent, cooling, aphrodisiac, antibacterial against Staphylococcus aureus and Escherichia coli, gonorrhoea, diarrhoea, dysentery, haemorrhoids and, anti-inflammatory, burns
2.	**Leaves and tender shoots**: Purgative, wounds, skin diseases
3.	**Leaf juice**: Asthma, cough, sexual disorders, diarrhoea, haematuria, toothache, migraine, eye troubles, gastric problems, scabies

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

Material and methods:-
Preparation of extract:-*Ficus religiosa* leaves was collected from local area and removed foreign particles. Wash the leaves with water to remove dirt and debris and dried at 30-40°C in an oven.

Grinding:- Grind the dried leaves into a fine powder using a mortar pestle or a grinder and stored the leaves powder into tight packet.(25)

Ethanolic extraction of plant leaves:-
10gm of leaves powdered was weighed properly and dissolved into 100ml of ethanol. Set up Soxhlet apparatus. place the plant material into the thimble of the Soxhlet extractor. Put 100ml of ethanol 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.(26,27)

Phytochemical analysis

Test for Glycosides
Take 1ml of plant extract was taken and add few drops of Sulphuric acid, formation of reddish precipitate confirmed presence of glycosides.

Test for Carbohydrate (Molisch's test)
Take 1ml of extract was 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. (28)

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

Test for Saponins

Take 1ml extract was taken and 5ml water was added to it, shaken well in test tube shaker. Presence of saponin was confirmed by lather formation.

Test for Tannins (Ferric chloride test)

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

Test for alkaloids (Dragendroff's reagent)

Take 1ml of plant extract add 5-6 drops of Dragendroff's reagent were added. Presence of alkaloid was confirmed by formation of creamish, brownish red, orange precipitate(29)

Phytochemical screening of *Ficus religiosa* plant extract

Tests	Procedure	Result
Glycosides	1ml plant extract+ Few drops of sulphuric acid	Reddish precipitate occurs
Carbohydrate	1ml extract+ 2ml Molisch's reagent+2ml conc. sulphuric acid	Formation of reddish violet ring at the junction of two liquids
Flavonoid	1ml plant extract+1ml aqueous NAOH	Formation of yellowish colour
Saponins	1ml extract+5ml water. Shake it properly.	Formation of foam for 10-15 min it shows the presence of saponins.
Tannins	1ml extract +1ml ferric chloride	Formation of greenish black colour
Alkaloids	1ml of plant extract + 5-6 drops of Dragendroff's reagent	formation of creamish/ brownish red/ orange precipitates.

Table2:- Phytochemical tests of *Ficus religiosa* leaves extract

Thin Layer Chromatography:

chromatography method for separating mixtures is called thin layer chromatography, or TLC. A sheet of glass, plastic, or aluminium foil is used for thin layer chromatography. The sheet is coated with a thin layer of an adsorbent substance, commonly cellulose, silica gel, or aluminium oxide (blotter paper). The stationary phase is the layer of adsorbent that is present here. Using thin layer chromatography, one can: 1. Watch the formation of a reaction; 2. Identify the compounds included in a particular sample and evaluate its purity.(30)

Plate preparation:-

A thick slurry of the two mixtures was spread out on TLC plates measuring 18.5 x 5 cm. By mixing water and silica gel powder in a 2:1 ratio (10g of silica gel powder in 20ml of water), the silica gel mixture is utilized as the adsorbent material. The resulting plate is heated to 110ºC for 30 minutes in order to dry and activate it.
For preparative TLC, the adsorbent layer is usually between 0.5 and 2.0 mm thick.

Preparing **Solvents**:-

The organic solvent that was used for TLC is produced by combining methanol and chloroform in a 9:1 ratio. Method: The steps listed below are followed in order to run a TLC:A small portion of sample-containing extract is placed into a plate at a distance of approximately 1.5cm from the bottom. If the solvent is not allowed to evaporate fully, there will be very little or no separation. Less than 1 cm of a prepared solvent is added to the separation chamber in a tiny amount. The chamber is closed sealed using a cover glass or any other type of lid, and the solvent vapours are allowed to fill the chamber for a few minutes. Next, the TLC plate is placed.

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*.(31,32)

1) Antibacterial activity

Aqueous and ethanolic extracts of *F. religiosa* leaves showed antibacterial effect against *Staphylococcus aureus, Shigella dysenteriae, S. typhimurium, Pseudomonas aeruginosa, Bacillus subtillis, 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*.(33) 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(34,35)

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.(36,37)

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 degranulates.(38)

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). 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.(39,40)

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.(41)

Chemicals and Reagents

Chemicals and reagents used for the study –Ethanol extract, Molisch's reagent, Dragendroff's reagent, Chloroform, Acetone ,Sulfuric acid, Aqueous NAOH, Ferrous sulphate, Distilled water, Ferric chloride . These reagents and chemicals were used in pure state. (42)

Microorganism

Four microorganisms representing Gram positive and Gram negative were used in this study. Two-gram positive bacteria were *Staphylococcus aureus* and *Bacillus subtilis* and two-gram negative bacteria were *Escherichia coli* and *Salmonella typhi*.(43)

Antimicrobial activity isolation of microbial strain:-

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus* species were the microorganisms used in the present study. To try to isolate these pathogenic microorganisms, one hundred midstream urine specimens from patients who were hospitalized in Hospital with urinary tract infections (UTIs) were collected. The microbiology department treated these specimens in accordance with standard procedures.(44) Most of the resistance strains were selected after the isolates' antimicrobial susceptibility patterns were determined using the Kirby Bauer disc diffusion method in accordance with CLSI 2013 recommendations. (45,46)Drugs used for *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus species* were Amoxicillin/Clavulanic acid (30µg), Ampicillin/Sulbactam (30µg), Cefepime (30µg), Cefotaxime (30µg), Cefuroxime (30µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cephalexin (30µg),), Cefaclor (30µg), Cefixime (30µg), Imipenem (10µg), Meropenem (10µg), Aztreonam (30µg), Amikacin (30µg), Gentamicin (10µg), Tobramycin (10µg), Doxycycline (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Ofloxacin (5µg), Norfloxacin (10µg), Moxifloxacin (5µg), Nitrofurantoin (300µg), Fosfomycin (50µg), Piperacillin/ Tazobactam (110µg), Colistin (10µg), Polymyxin B (300µg), Tigecycline (15µg) and Ticarcillin/Clavulanic acid(85 µg). Selected strains of *Escherichia coli*, *Pseudomonas aeruginosa*, *Kleibsiella pneumoniae* and *Proteus species* were only sensitive to Fosfomycin, Colistin, Polymyxin B and Tigecycline only.(47)

Diffusion method

The agar well diffusion method was used to measure the antibacterial activity. On an agar plate, 100 µl of cultures of *S. aureus* and *E. coli* were spread out. After opening four wells and filling them with 50 µl of sample extracts, the mixture was allowed to further disperse and was then incubated for eighteen hours at 37°C. Zone of inhibitions was measured on the next day. The antibacterial activity test was based on the measurement of the diameter of the zone of inhibition formed around the well.(48,49)

Finding the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC)

The plant extracts that showed the greatest antimicrobial activity against most of the microorganisms tested in the disc diffusion assay were

further tested for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), despite the fact that the results of the disc diffusion assay cannot always be compared to the minimum inhibitory concentration (MIC) data.(50) Eloff described the micro dilution approach utilizing a 96-well microplate to determine the minimum inhibitory concentration (MIC) and minimum bactericidal count (MBC) of the crude extracts against these bacteria. Select bacteria from the standard 0.5 McFarland solution were introduced to 96-well microplates that had been serially diluted twofold for a concentration range of 100 µg/dl to 800 µg/dl using sterile distilled water.(51)

ABBREVIATIONS:-

UTI	Urinary tract infection
MIC	Minimum inhibitory concentration
MBC	Minimum bacterial concentration
TLC	Thin layer chromatography
NAOH	Sodium hydroxide

CONCLUSION:-

The whole world is filled with an abundance of medicinal plants. For a wide range of functions, people living in poor countries with poor health facilities depending heavily on medicinal plants. The main goal of this study was to identify the bioactive ingredients found in the *F. religiosa* extracts as well as their antimicrobial properties against *S. aureus* and *E. coli*. The experiment used four distinct extracts of the *F. religiosa* leaf: aqueous, hydro-alcohol, methanol, and acidified water. The results showed that all of the extracts contained tannin, resin, and alkaloids. Flavonoids or cardiac glycosides are absent in this study. The treatment of serious, life-threatening illnesses would greatly benefit from additional research on the identification, pharmacodynamic analysis, and isolation of effective antimicrobial compounds from medicinal plants. Medicinal plant extract used for various disease. It does not show any sides effects. In this time wide range of people used ayurvedic medicines. From the study we conclude that most of the plants showed antimicrobial activity against one or more UTI isolates. Broad spectrum activity was observed in *Ficus religiosa*. The aqueous and ethanolic solvent extract was used to screen the secondary metabolites and test the antimicrobial effect of extract on *E.coli*. The phytochemical analysis showed the presence of alkaloids, saponins phenols flavanoids protein tannins and terpenoids. The extracts were subjected for antimicrobial activity against *E.coli* using agar well diffusion method. Aqueous and methanolic extract showed a zone of inhibition.

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