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Investigation of Herbal Oral Rehydration Solution [HORS] with Antibacterial Potential



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ABSTRACT

The bark of *Ficus religiosa* was studied for *in vitro*, *in vivo*, phytochemical screening and antimicrobial activity. The aqueous and methanolic solvent extract was used to screen the secondary metabolites and test the antimicrobial effect of extract on *E. coli* and *Stapylococus aureus*. The phytochemical analysis showed the presence of some phytoconstituents, these are phenols, alkaloids, saponins, protein, tanins, flavanoids, and terpenoids. The extracts were subjected for antimicrobial activity against *E. coli* using agar well diffusion method and castor oil induced method. Methanolic and aqueous extracts showed a zone of inhibition of 10 mm and 12 mm respectively.

1. INTRODUCTION:

DEHYDRATION: The word Dehydration means a loss of body fluids, which having of water and salts. Large amounts of water and salts lose from bodies in case of diarrheal condition, and body become dehydrated very quickly. Dehydration condition is very dangerous, especially for babies and toddlers. Children can die if they not treated early.

Dehydration is not always related to this condition, it may be caused by:

- Vomiting & diarrhea.
- Drug that increase urine excretion (diuretics).
- Excessive sweating.
- Decreased water intake.
- Burns.
- Heat.

Signs of dehydration:

□ Low urine output (less than four wet diapers in 24 h)

 \Box No tears

- \Box Dry skin, mouth and tongue
- □ Sunken soft spot (fontanel) on infant's head and sunken eyes
- □ Grayish skin

DIARRHOEA: Diarrhea is a very common problem occurs in childhood. Sometimes it is mild and brief. It can be very severe, especially in infants. The symptoms of diarrhea is the passage of three or more loose watery stools. It is characterized by increased gastrointestinal motility and a decrease in the absorption of fluid and electrolytes. Diarrhea is classified into 3 type based on the duration:

- 1. Acute diarrhea (duration < 2 weeks).
- 2. Persistent diarrhea (duration from 2 to 4 weeks).



3. Chronic diarrhea (duration of more than 4 weeks).

Signs of Diarrhea:

The main symptom of diarrhea is watery stool with cramps, but it may be accompanied by some other symptoms. These are:

- Stomach pain
- Abdominal cramps
- Bloating
- Weight loss
- Fever
- Body aches
- Chills

Diarrhea is very dangerous in some times and other possible symptoms are very serious:

HUMAN

- Blood or pus in the stool
- Persistent vomiting
- Dehydration

Ficus religosa linn as herbal medicine:

Ficus religosa Linn is used in traditional system of medicine for the treatment of several disorders and is one of the herbs mentioned in all ancient scriptures of Ayurveda, Siddha, Unanni and Homeopathy. *Ficus religosa Linn. (Family; Moraceae)* is used in traditional system of medicine for the treatment of several disorders. Various plant parts such as bark, root , leaf , fruits and latex are used as astringent, diarrhea, diabetes, haemoptysis, carminative etc. Many plants have played a significant role in medicinal value it helps in maintaining human health and also help to improving the quality of human life for thousands of years. The containing of various phytochemicals it is used for treatment many of illness. Focus on plants research has increased all over the world and a large body of

evidence has collected to show immense potential of medicine plants used in various traditional system.

2. PLANT PROFILE:

Plant to be used – Ficus religosa

HISTORY:

Ficus religiosa, commonly known as Peepal, is the belongining in the most family Ficus and it is known by in excess of 150 names. *Ficus religiosa* has strict, legendary and therapeutic significance in Indian culture. References to *Ficus religiosa* are found in few antiquated sacred writings like Arthasastra, Puranas, Upanishads, Ramayana, Mahabharata, Bhagavad-Gita and Buddhistic writing and so forth. *Ficus religiosa* is also known as the Bodhi tree, even before Gautama Buddha sat under its branches reflecting and accomplished in lightenment. This plant is viewed as sacrosanct by the adherents of Hinduism, Jainism and Buddhism, and consequently the name was given to it. Siddhartha Gautama is alluded to have been sitting underneath a Bo Tree when he was & quot; edified & quot; (Bodhi) or & quot; stirred & quot; Thus, Bo Tree is notable image for satisfaction, thriving, life span and good karma.[1] *Ficus religosa* tree was found in all over asia. These are commonly and easy to available types of tree they having many of medicinal property. Peepal tree is esteemed with various vernacular names. [1]

VERNACULAR NAME:

Sr.	T	Vernacular Name	
No.	Language		
1.	Hindi	Peepal	
2.	English	Bo tree, Peepal tree, Bodhi, Sacred tree	
3.	Telgu	Ravichettu	
4.	Gujrati	Piplo, jari, pipers, piparo	
5.	Bengali	Asvattha, ashud	
6.	Malayalam	Arrayal, ashwarthan, arasamaram, arasan	
7.	Tamil	Ashvattha, bodhidruma, pippala, vrikshraj	
8.	Marathi	Papal, pimpal	

Table No. 1: Vernacular Names of Ficus religiosa

DISTRIBUTION:

Ficus religiosa is found to the Asia-Tropical regions including india, Pakistan, China, Myanmar, Thailand, Vietnam, Iraq, Bangladesh and Nepal. The plant natively belongs to tropical Asia from where it has now been introduced, spread and cultivated throughout everywhere of the world. Ecologically, it is scattered in forests, where it propagates as an epiphyte on other trees. Apart from the wide distribution of *Ficus religiosa*, it is endured with more than 150 names around the world.

Table No. 2: Wordly identities of Wide distribution of "Ficus religiosa"

Sr. No.	Country	Popular name in Country
1.	India	Arachu, arasu, peepal, ashathwa, bodhi
2.	French	Arbre bo, arbre de dieu
3.	Cuba	Alamo
4.	Spanish	Higuera de agua
5.	Chinese	Puti shu
6.	Brazil	Figueira religosa

Synonymes- Pippal, Bobhi, Wisdom, Goolar.

Biological Sources - These are the dried leaves, bark and fruits of *Ficus religiosa* belonging to family moraceae. [6]



Fig. No. 1.1: Ficus religosa

Scientific classification:

Kingdom: Plantae Clade: Tracheaphytes Clade: Angiosperms Clade: Rosids Order: Rosales Family: Moraceae Genus: Ficus Subgenus: F. subg. Urostigma Species: *F. religosa*

Morphology:

This large and old tree and the height of this tree is of 30m long. They break bark and are of white or dark colored in shading. The leaves of the plant are slim, glossy and bear 5-7 veins. Natural products are little, about ¹/₂ crawls in breadth, like that of eye student. At the point when it is crude, it is of green shading and turns dark when it is ready. The tree natural products in summer and the organic products get aged by stormy season. [5, 6]

Leaves – the leaves of plant shading is red-pinkish, yet then they turn dark green and develop to around 12 to 18 cm long (5-7 inches). They are joined to long adaptable stalks which makes them stir, shudder and move in the smallest whiff of wind.

Bark – Bark having in level or marginally bended pieces, shifting from 1.0 to 2.5 cm or more thickness. External surface darker or debris shaded, surface lopsided because of peeling of stopper.

Flower - The little red blossoms show up in February. The colour of flower is yellow to red. Fruit– The fruit of plant blowing in may to june. Size is 12, 13 mm or ½ inch in width, which shows up two by two in the edges of the leaves on the twigs. Chemical constituents: Preliminary phytochemical screening of Ficus religiosa bark showed the presence various phytoconstituents and these are flavonoids, steroids, terpenoids, cardiac glycosides, tannins and saponins. The bark of *Ficus religiosa* showed the presence of bergaptin, bergaptol, lanosterol, β -sitosterol, stemasteddatrol, lupen 3 one, β sitosterold glycosides, vitamin-K the bark also contain tannin wax saponin β-sitosterol leucocyanidin-3- $0-\beta$ -d-glucopyranncoside, and leucocyanidin-3-0- α -d-glycopyranoside, leucopelargonidinluperol acetate, and α -amyrin acetate eucoanthocynidin and leucoanthocynin leave yield campestrol. The crude latex of Ficus religiosa shows the presence of a serine protease named religiosin is an acidic protein acts at pH 8-8.5 and temperature 5^oC. Reverse phase high performance liquid chromatography flavonoids in *Ficus* religiosa using amnetin, myricetin, is or and quercetin as standards.[7]

Pharmacological activity of Ficus Religiosa:

Ficus Religiosa tree occupy various pharmacological efficacy all over part of the plant use for medicinal purpose the various part of the tree is fruit, bark, Leaves, flower, root and latex all parts of tree are useful in the treatment of various disorders. It is also used in combination with other compounds/plant extract (10, 11, 12). Some various pharmacological activity of tree discussed in below:

1. Ficus religiosa possess anti-diabetic activity-

According to several researchers reports the bark of the tree acquire anti-diabetic activity. The reports possess that orally taken of bark extract of tree at proper dose level (25mg/kg, 50mg/kg, 100mg/kg) streptozotocin (STZ) induced diabetes in rat is effective against diabetes. After the administration of extract, they observed that the significantly decrease the level of glucose in rat model and induce insulin level in liver and also useful for decrease the triglyceride level and cholesterol level.

2. Anti-inflammatory Activity of Ficus religiosa-

The bark extract (methanolic) of *F. religosa* having anti-inflammatory activity and also having analgesic activity. The study shown that methanolic extract of *Ficus religiosa* administered in carrageenan induced paw edema in animal model proper dose level at proper dose level (125mg/kg, 250mg/kg, and 500mg/kg).

3. Analgesic Activity of Ficus Religiosa [13]-

Some research reports shown that the methanolic extract of bark *Ficus religosa* possess analgesic activity. Writhing test (It is a chemical method test is used to induce a pain in animal model) using acetic acid in rat this test is used for testing of analgesic potencial of plant. At required dose level (125mg/kg, 250mg/kg, 500mg/kg) and at 250mg/kg the extract was taken orally for shown analgesic activity which is similar to aspirin and indomethacin (analgesic activity possess drug).

4. Anti-bacterial effect of Ficus religiosa (11)-

Ficus religiosa carry inhibitory activity against many bacteria includes *Staphylococcus aureus*, *Salmonella paratyphi*, *Escherichia coli* (E. coli), *Shigella dysenteriae*, *Bacillus subtillis*, *Pseudomonas aeruginosa*. In Evaluation of research possess that the aqueous and ethanol extract of leaves of *Ficus religiosa* possess anti-bacterial activity. And choloroform extract of leaves also having potent anti-bacterial activity against many bacteria.

5. Anti-microbial activity of Ficus religiosa (11)-

Some research and studied shown that the plant of *F. religosa* also possess anti microbial activity against many microbes such as *Bacillus megaterium* (rod like, gram positive bacteria), *Azobacter chroococcum*, *B. cereus* (gram positive bacteria), *Streptococcus faecalis*, *Streptomycin lactis*, *Klebsiella pneumonia* etc. In investigation of study found that the chloroform extract of fruits is effective against some microbes.

3. MATERIAL AND METHODS -

1. Collection of Sample:

Bark of *Ficus religiosa* was collected from the campus of School of Pharmacy, Chouksey Engineering College, Bilaspur [C. G.]. And this collected bark was thoroughly rinsed with distilled water and shade dried in the campus aboratory.

2. Preparation of extracts:

Dried Bark was then grinded into fine particles with the help of grinder, further stored into air tight packets.

Distilled water extract (aqueous extraction): Take 5gm of powdered leaves was taken in small conical flask. Then 50ml of distilled water added. Further flask was kept on the rotary shaker at 200 rpm for 24hrs.

Soxhlet apparatus *extraction (solvent extraction):* About 50 gm of the dried bark powder will be successively extracted with solvent of increasing polarity such as petroleum ether, chloroform, ethyl acetate, methanol, and ethanol:water [50:50] for 24 hour with each solvent, using the Soxhlet apparatus as a temperature of 35° c each time before 50° c and then subjected to further extraction. The concentrated extract will be reduced to a semisolid mass by drying on a water bath at $40^{\circ} + 5^{\circ}$ c and pack into separate air- tight container.



Fig. No. 1.2: Soxhlet apparatus

PHYTOCHEMICAL SCREENING-

The quantitative assay are performed for detection of primary and secondary metabolites present in plant bark it was performed using standardized methods for the phytochemical analysis of the plant extracts.

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Phytochenical	Aqueous extract	Methanolic extract	
Alkaloid	-ve	-ve	
Carbohydrates	+ve	+ve	
Saponins	+ve	+ve	
Phenols	+ve	+ve	
Flavonoid	+ve	+ve	
Protein	+ve	+ve	
Tannins	+ve	+ve	
Terpenoids	+ve	+ve	

Table No. 3: Phytochemical screening of *Ficus religosa* bark extract

When +ve shows presence and -ve shows absence of phytochemical in the sample.

4. IN-VITRO STUDY:

4.1 MICROBES USE: Two Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*), one Gram-positive bacteria (*Staphylococcus aureus*), and one fungus (*Aspergillus niger*) will be collected. All the bacterial samples will be maintained in nutrient agar slants. The fungus sample will maintain in PDA medium plates.

4.2 Screening of antibacterial and antifungal activity: In vitro antibacterial and antifungal studies will be conducted against selected bacteria and fungus using the disc diffusion method. The different concentrations of extracts (100, 200, 300 & amp; 400mg/ml) of bark will be prepared. The Whatman no: 1 filter paper disc size of 30 mm is used for the disc diffusion method. Paper discs are incubated in plant extracts overnight at room temperature and air-dried before being used in the experiments.

5. In Vivo Study

5.1 Animal

Albino Wistar rats (175 - 200 g) of either sex are obtained. The animals will be maintained in a well-ventilated room with a 12:12 hour light/dark cycle in polypropylene cages. The animals will feed with a standard pellet diet and water *ad libitum*. Animal Ethical Committee clearance will be obtained from the Institutional Animal Ethics Committee.

5.2 Castor oil-induced diarrhoea

Rats will be fasted 24 hours before the test with free access to water *ad libitum*. Rats will be treated orally with vehicles and selected extracts of *Ficus religiosa Linn*. (Stem bark extracts, 200 mg/kg, p.o) and standard drugs (loperamide, 3 mg/kg, p.o.). One hour after drug treatment, each rat receives castor oil (1 ml each orally). Each rat is then housed separately in a cage over clean butter paper. Then diarrhoeal episodes are observed for a period of 4 hours. During this period, the consistency of feces, total number of diarrhoeal feces within 4 hours, and total weight of feces after 4 hours will be recorded.

6. DRUG AND CHEMICALS:

Castor oil (Patanjali ayurvedic LTD), 0.9% sodium chloride (normal saline) (enzymes pharmaceuticals, surar), gum acacia, and loperamide (Lupin ltd. Mumbai).

4. RESULTS AND DISCUSSION:

1. EXTRACTION OF PLANT MATERIAL -

Preparation of bark extract of Ficus religosa linn. were done by Soxhlet extraction method.

Soxhlet Extraction

A Soxhlet extractor having main three primary segment: a thimble (typically made of thick filter paper) which retains the solid to be extracted, a percolator (boiler and reflux) which flows the dissolvable and a siphon instrument, which occasionally discharge the thimble.

Assembly

- 1. The source material containing the compound to be separated is put inside the thimble.
- 2. The thimble is staked into the fundamental office of the Soxhlet extractor.
- 3. The extraction dissolvable to be utilized is set in a refining cup.
- 4. The flask is placed on the heating mantle.
- 5. The Soxhlet extractor is set on the cup.
- 6. A reflux condenser is set on the extractor.

Procedure

About 50 gm of the dried bark powder will be successively extracted with solvent of increasing polarity such as petroleum ether, chloroform, ethyl acetate, methanol, and ethanol: water [50:50] for 24 hours with each solvent, using the Soxhlet apparatus as a temperature of 35'c each time before 50'c and then subjected to further extraction. The concentrated extract will be reduced to a semisolid mass by drying on a water bath at 40' +_ 5'c and pack into separate air- tight container.



A. Extraction process

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B. extract

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Fig. No. 1.3: Soxhlet apparatus

2. Phytochemical screening-

Quantitative assay was performed for detection of presence of plant primary and secondary metabolites by using standardized methods for the phytochemical analysis of the plant extracts.

1. Detection of alkaloids- 1ml of aqueous extract was stirred and placed in 1% of aqueous hydrochloric acid on a steam bath. Then take 1 mL of the filtrate and treated with Dragendorff's reagent. Turbidity or precipitation with this reagent was considered as evidence for the presence of alkaloids [16].

2. *Detection of carbohydrates-* Benedict's test– Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate [17].

3. *Detection of saponins-* 2 g of the powdered sample was taken and boiled in 20 ml of distilled water in a water bath than filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. Then add 3 drop of olive oil and shaken continuously, then observed for the formation of emulsion [18].

4. *Detection of phenols*- Extracts were treated with 3-4 drops of ferric chloride solution. Bluish black color was obtained that indicates the presence of phenols [19].

5. *Detection of flavonoids*– Take a portion of the powdered bark sample with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and placed 4 ml of the filtrate mixed with 1 ml of dilute ammonia solution and shake properly. A yellow colouration was observed indicating a positive test for flavonoids becomes colourless on addition of dilute acid, indicates the presence of flavonoids [18].

6. Detection of proteins- Take extract solution and treated with ninhydrin reagent (2, 2dihydroxyindene-1, 3-dione) was added and boiled for few minutes. Blue color was formed this indicates the presence of amino acid.

7. *Detection of tannins*- 0.5 gm of the extract solution was boiled in 10 ml of water in a test tube and then it filtered. Then add few drops of 0.1% ferric chloride and observed for brownish green or a blue-black colouration [18].

8. *Detection of terpenoids* - 5 ml of each extract were mixed in 2 ml of chloroform and 3 ml concentrated sulphuric acid was carefully added to form a layer. A reddish brown colour at the interface indicates the presence of terpenoids [19].

Phytochemical screening: Phytochemical screening of plant extract in methanolic extract and aqueous is shown in table 4. The aqueous extract showed the presence of carbohydrates saponins, phenols, flavonoids, tanins and terpenoid except alkaloid. While methanol extract showed presence of all phytochemical mentioned in table 3.

Phytochemicals	Aqueous extract	Methanolic extraxt
Alkaloid	Negative	Positive
Saponins	Positive	Positive
Tannins	Positive	Positive
Terpenoids	Positive	Positive
Phenol	Positive	Positive
Flavonoid	Positive	positive
Carbohydrate	Positive	Positive
Protein	Positive	Positive

Table No. 4: Phytochemical screening of crude extracts of Ficus religiosa

Where positives = presence of phytochemical

Negative =absence of phytochemicals

2. Acute oral toxicity study:

Acute oral toxicity test was performed based on the organization for economic cooperation and development (OECD) guideline, number 425. All animals were observed continuously for toxicities like diarrhoea, decreased of appetite, hair erection and loss, lacrimation, salivation for the first 1 hour continuously and intermittently for the next 3 hour and periodically for 24 and later cage side observation continued for 14 days (25).

3. In-vitro study –

Agar well diffusion method:

The antimicrobial activity of the crude root extract of *Ficuus religosa* was done according to Umer *et. al.*, and Molla *et. al.* (26, 27). It was performed on both American type cell culture (ATCC) and clinical isolated of selected intestinal pathogens obtained from hospital, which were considered as a major cause of diarrhoea such as *Shigella flexneri* (*S. typhi*), *Salmonella species*, *E. colli*.

Three test concentrations of plant extract (800 mg/ml, 400mg/ml and 200mg\ml), vehicle and standard antibiotic disc was used for determination zone of inhibition. Loperamide were used as a standard drug and distilled water was used as a negative control. The standard antimicrobial discs were selected based on the susceptibility of bacterial species (23). The

antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism by using vernier caliper and then compared with the reference as well as the control. Each procedure was done in triplicated and average values were being taken for further use (26, 27).

4. In-vivo study -

Animal handling –

Albino wistar rates (175 -200gm) of either sex were used for the experiment. The animal were obtained from and maintained in the laboratory of Department of Pharmacology and Toxicology, School of Pharmacy, Chouksey Engineering College. All animals were housed in plastic cages at room temperature in an air – conditioned room of 12 hour light/dark cycle with accesses of pellet diet and clean water *ad libitum*. Before any experiment was started all required animals were allowed a week of accommodation to the experimental environment and all the experiments were carried out by following the internationally accepted laboratory animal care and use guideline (25). Animal Ethical Committee clearance will be obtained from the Institutional Animal Ethics Committee.



Fig. No. 1.4: Handling of animal

Grouping and dosing of animal -

Rats were randomly assigned into four groups of four animals each to perform antidiarrhoeal activity using castor oil –induced model. The negative control group treated with distilled water (10ml/kg), positive control groups received the standard drug loperamide (3mg/kg) in experiment. The two tested dose was selected based on the result of acute toxicity study.

Based on that 200 and 400 mg/kg were considered as low dose, middle dose and high dose, respectively.



Fig. No. 1.5: Grouping and dosing of animal

Castor oil induced model -

Rat of both sexes (95-100 g) were fasted for 18 hour. Then the selected rats for experiment were divided into four group (n=5). Group 1 was given normal saline (2ml/kg) orally as control group and group 2 received loperamide (3mg/kg) as standard group. Group 3 & 4 received *Ficus religosa* bark extract (200 and 400 mg/kg). After 1 hour, all groups received castor oil 1ml each orally. Then all animal groups were placed in cages and lined with adsorbent paper and observed for 4 h for the detection of characteristic diarrhea dropping. 100% was considered as the total number of feces of control group. (26, 29). And the activity was expressed as % inhibition of diarrhea. The (%) inhibition of defection was measured using the following formula:

Average number of WFC – average number of WFT %Inhibition = ------ × 100 Average number of WFC

Where, WFC= average number of wet faeces in the control group.

WFT = average number of wet faeces in the test group.

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Fig. No. 1.6: Dose administration (Castor oil- induced diarrheal)

Table No. 5: Effect of *Ficus religiosa* bark extract on castor oil- induced diarrhea in rats.

GROUP	TREATMENT	TOTAL NO. OF FECES	% INHIBITION OF DEFECATION	TOTAL NUMBER OF DIARRHEAL FECES	% INHIBITION OF DIARRHEA
1.	Castor oil + saline (2ml/ kg p.o.)	17.18±1. 91		10.05±1.08	_
2.	Cator oil +loperamide (3mg/kg i.p.)	6.76±0.66	56.32	5.00±0.33	53.75
3.	Castor oil + bark extract (200mg/kg i.p.)	10±0.81	43.98	6.22±098	43.67
4.	Castor oil +bark extract (400mg/kg i.p.)	8±1.25	54.55	5.70±0.52	57.75

Values were expressed as mean \pm SEM (n= 5) *p<0.05, **p<0.01 when compared with control group.

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Fig. No. 1.7: Effect of *Ficus religiosa* bark extract on castor oil- induced diarrhea in rats.

RESULTS:

The crude root extract of *Ficuus religosa* showed a dose dependent response in prolonging the onset of diarrhoea. As shown in table 3, the onset of diarrhoea was significantly protected at doses of 200 and 400 mg/kg as compared to the negative control. In addition, the largest test doses have shown maximum prolongation in diarrhoea onset as compared to the standard drug loperamide 3mg/kg. All the dose of the crude bark extract of wet faces as compared to the negative control.



Fig. No. 1.8: Weighing of Faeces

5. CONCLUSION:

The finding of the present study provide convincing evidence that methanolic extract of *Ficus religosa* bark have remarkable antidiarrheal activity and its also show rehydration property. Antidiarrheal effect is rapid, rehydration property is excellent, long lasting, and statistically significant at both 200 and 400 mg/kg doses.

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