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Original Article

Preliminary phytochemical screening of bark (powder) extracts of *Ficus religiosa* (peepal) plant

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ABSTRACT: The traditional medicine involves the use of various different plant extracts or the bioactive constituents. The study such as ethno medicine keenly represents the best avenues in searching new economic plants for medicine. This type of study gives the health application at affordable cost. The present study carried out to find out the phytochemical constituents in the *Ficus religiosa* bark. The *Ficus religiosa* was collected from the Rama University Campus. The shadow dried bark materials were grained and extracted with petroleum ether, chloroform, methanol and ethanol: water (50: 50). Photochemical analysis was carried out according to standard procedures. The bark powder was successively extracted with Phytochemical screening shows the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, phytosterol, tannin & flavonoids. The result of the study could be useful for description and phytochemical analysis of the plant.

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INTRODUCTION

Peepal (*Ficus religiosa*), is a large tree in "Moraceae" family, traditionally used in treatment of various types of diseases like (diabetes, menstrual disorders, washing ulcers, leucorrhoea, erysipelas, antibacterial and antifungal)[1]. The present study was carried out to investigate the phytochemical profile of bark of *Ficus religiosa*[2,3]. The bark powder was successively extracted with petroleum ether, chloroform, methanol and ethanol: water (50: 50). Phytochemical analysis shows the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, phytosterol, tannin & flavonoids[4]. The result of the present study could be useful for description and foundation of monograph of the plant.

The Pharmacognostical studies are one of the major criteria for identification of herbal drugs from plants. Medicinal plants form a large group of economically important plants that gives the basic raw materials for indigenous pharmaceuticals[5-7]. One approach to the discovery of new drugs is the study of the bioactive constituents of higher plants.

The investigation of plants used as remedies in the traditional folk medicine can be an interesting tool to identify several biologically active molecules from the 250,000 higher plant bioactive constituents with antiinflammatory, analgesic, antipyretic and anti-ulcerogenic activity[8-10]. *Ficus religiosa* (*F. religiosa*) commonly known as peepal is a very big sacred tree and found throughout India in the vicinity of temples.

MATERIALS AND METHOD:

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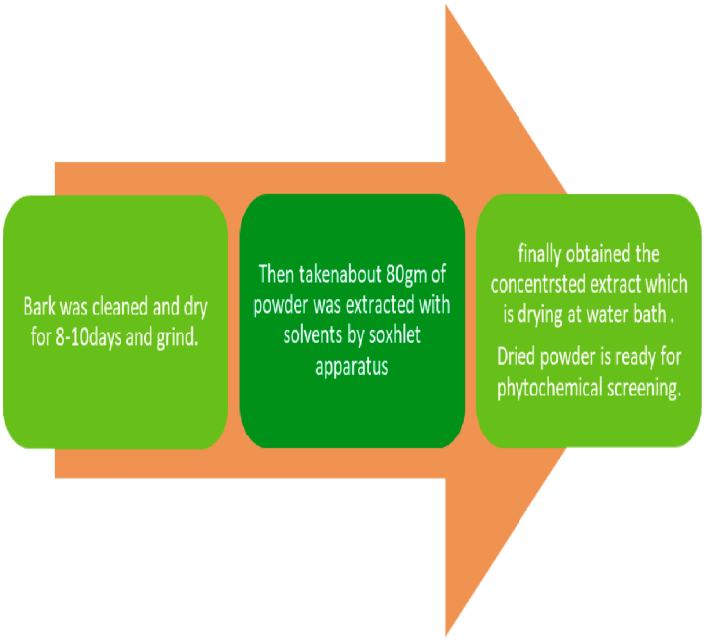
Collection of plant materials: The fresh bark of *Ficus religiosa* plant was collected in the month of October from Kanpur district, Uttar Pradesh. The plant was authenticated by Dr. Rishikesh Gupta (Associate Professor), Bundelkhand University Jhansi.

The bark of *Ficus religiosa* were cleaned,cutted into small pieces and dried under shade at room temperature (37°C) for one week. The dried barks were powdered and stored in air tight containers for the phytochemical investigation.

Preparation of the extract:

- The bark of *Ficus religiosa* was cleaned and shade dried in open air for 8-10 days then pulverized to dry power using electric grinder.
- About 50 gm of the dried bark powder was extracted with hot solvents 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 at a temperature of 30 to 35˚C.
- Each time before extracting with next solvent, the powdered material was air dried below 50˚C and then subjected to further extraction.
- The concentrated extract was reduced to a semisolid mass by drying on water bath at 40±50˚C and packed into separate air tight containers.
- These extracts were subjected to phytochemical screening for the identification of the different phytoconstituents.



The percentage extractive yield was calculated by formula as mentioned below:

% Extractive yield (w/w) = weight of dried extract ÷ Weigh of dried bark × 100

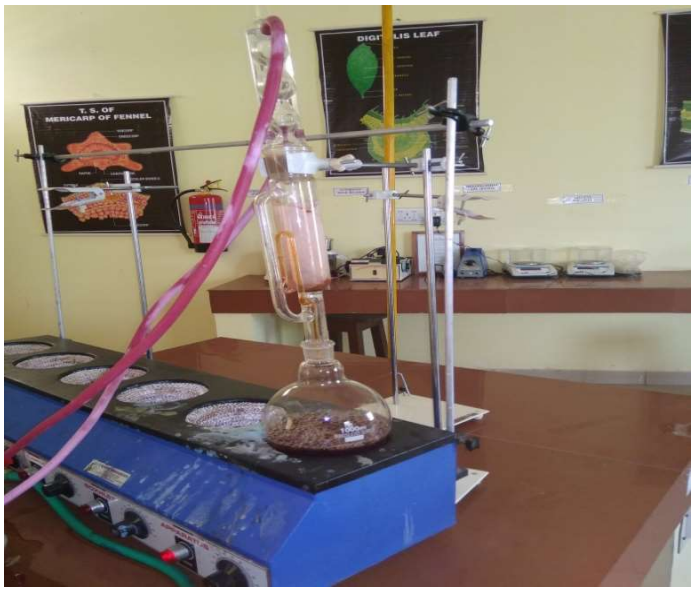


Fig. 1: Extraction of *ficus religiosa* bark powder

DETERMINATION OF EXTRACTIVE VALUE:

The extractive values of dried bark powder of fig. religiosa were determined with different solvents i.e. petroleum ether, chloroform, methanol, and **ethanol: water is (50:50)** and water.

Preliminary physical analysis of dried bark extract:

The property of selective reactivity of phytochemical present in an extract forms the basis of chemical tests for identification of different chemical constituents[11,12]. Preliminary analysis of *Ficus religiosa* bark extract was performed initially to identify various chemical compounds present and to assess physicochemical properties. The performed preliminary analysis included:

a**) Macroscopic evaluation of bark extract:**

Macroscopic evaluation of bark extract was performed with respect to:

Sr. No.	Macroscopic parameters	Result
1	Colour	Brown
2	Odour	Characteristics
3	Taste	Bitter
4	Touch	Fine dry powder
5	Appearance	Powder

b) Analysis of solubility parameters:

Solubility of prepared bark extract of *F. religiosa* was determined in various solvents such as:Distilled water, Methanol, Ethanol, Benzene andChloroform.

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c) Preliminary phytochemical screening:

The various extracts of *Ficus religiosa* i.e. Petroleum ether, chloroform, methanol and ethanol: water (50:50) was subjected to qualitative chemical analyses to detect the presence of various phytoconstituents[13-15].

1. **Tests for Carbohydrate[16,17]:** A small quantity of the extract was dissolved separately in 5 ml distilled water and filtered. The filtrates were subjected to the following tests to detect the presence of carbohydrates.

a) Molisch's test:

Step-1. The first step is to take extract filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube separately and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes[18].

Step-2. Formation of violet ring at the junction may indicate the presence of carbohydrates.

2. **Test for reducing sugar:**

a) **Fehling's test:**

Step- 1. The Extract filtrates were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately[19].

Step-2. The mixtures were boiled for 5-10 minutes on water bath. Reddish brown colour was obtained due to formation of cuprous oxide which indicated the presence of reducing sugar.

b) **Benedicts test:**

Step-1. Extract filtrates were treated with equal volumes of Benedict's reagent in test tubes separately[12,13].

Step-2. The mixtures were boiled for 5-10 minutes on water bath. Solution appeared green, yellow or red depending on amount of reducing sugar present in each filtrate[2].

3. **Test for Glycosides:**

a) Test for cardiac glycosides:

Keller kelliani test (test for deoxysugar):

Step-1. Bark were treated with chloroform and evaporate it to dryness.

Step-2. Separately 0.4 ml of glacial acetic acid containing a trace amount of ferric chloride was added and transferred to a small test tube added with carefully 0.5 ml of concentrated Sulphuric acid by the side of the test tube, blue colour appears in the acetic acid layer indicating the presence of glycosides.

b) Test for Anthraquinone Glycosides:

Borntrager's test:

Step-1. Bark extract were boiled with 1 ml of dilute Sulphuric acid in a test tube separately for 5 min, filtered while hot, pipette out the supernatant or filtrate, cooled and shaken with an equal volume of dichloromethane[20,21].

Step-2. The lower levels of dichloromethan separated and shaken with half its volume with dilute ammonia.

Step-3. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides.

c) Test for Saponin Glycosides:

Froth test: - Bark extracts were treated with water in a semi-micro tube separately shaken well. The froth appeared thus indicating the presence of glycosides[6,7].

4. **Tests for Amino acid and Protein:**

a) Biuret test (General test):

Step-1. Bark extract were treated with 1 ml 10% sodium hydroxide solution separately and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added[22].

Step-2. The formation of purplish violet colour may indicate the presence of proteins.

b) Million tests (for proteins):

Step-1. 3 ml test solutions were mixed with 5 ml Million's reagent separately.

Step-2. White precipitate was formed which on heating turned to brick red. It may indicate the presence of amino acids.

5. **Tests for Sterols and Triterpenoids:**

a) Libermann-Burchard test:

Step-1. Bark extract were treated with few drops of acetic anhydride separately. The boiled and cooled, concentrated sulphuric acid was added from the side of the test tubes[22].

Step-2. A brown ring at the junction of two layers and the upper layer turning green which indicated the presence of sterols while formation of deep red colour indicated the presence of triterpenoids[22,23].

b) Salkowski's test:

Step-1. Bark extract were treated in chloroform separately with few drops of concentrated sulphuric acid, shaken well and allowed to stand for some time, red colour appeared in the lower layer indicated the presence of sterols while the formation of yellow coloured lower layer indicated the presence of triterpenoids.

6. **Tests for tannins and phenolic compounds:**

a) **Ferric chloride test:**

Step-1. Small amount of bark extract was shaken with water separately and warmed.

Step-2. Then about 2 ml of 5% ferric chloride solution was added and observed for the formation of green or blue colour which may indicate the presence of phenols[23,24].

b) Gelatin test:

Step-1. 1% gelatin solution containing 10% sodium chloride was added to bark extract.

Step-2. The Formation of precipitate indicated the presence of tannins and phenolic compounds[10].

c) Iodine test:

Step-1. Bark extract were treated with diluted iodine solution separately.

Step-2. Appearance of transient red colour indicated the presence of tannins and phenolic compounds.

d) Nitric acid test:

Step-1. Bark (powder) extract was treated with dilute nitric acid separately.

Step-2. Formation of reddish to yellowish colour indicated the presence of tannins and phenolic compounds[24].

7. **Test for alkaloids:**

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Step-1. About 500 mg of the bark (mixture) extract were stirred with about 5 ml of dilute hydrochloric acid separately and filtered.

Step-2. Each filtrate was tested with the following reagents:

a) **Dragendroff's test:**

Step-1. Few drops of Dragendroff's reagent (solution of potassium bismuth iodide) were added to each filtrate and observed for the formation of orange yellow precipitate which may indicate the presence of alkaloids[21,25].

b) **Mayer's test:**

Step-1. Few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to each filtrate and observed for the formation of white or cream colour precipitate which may indicate the presence of alkaloids[24].

c) Hager's test:

Step-1. Few drops of Hager's reagent (saturated aqueous solution of picric acid) were added to each filtrate and observed for the formation of yellow precipitate which may indicate the presence of alkaloids.

d) **Wagner's test:**

Step-1. Take few drops of Wagner's reagent (solution of iodine in potassium iodide) were added to each filtrate and observed for the formation of reddish-brown precipitate which may indicate the presence of alkaloids[24].

7. Tests for flavonoids:

a) **Shinoda test (Magnesium Hydrochloride reduction test):**

Step-1. To bark extracts, 5ml. 95% ethanol was added separately. Each mixture was treated with 0.5g magnesium turnings and few drops of conc. HCl.

Step-2. If produced the pink colour, confirm the presence of flavonoids[24,25].

b) **Alkaline reagent test:**

Step-1. Small quantity of each extract sample was taken and added with lead acetate solution.

Step-2. After few minutes, appearance of yellow colour precipitates which indicated the presence of flavonoids[25].

Fig. 2: Extracts of different solvents

Table 1: Phytochemical Screening

Solvent	Colour of extract	Odour	Consistency	Sense of touch	Amt. of extract (g)	% yield
Petroleum ether	Brownish dark green	Characteristic	Semisolid	Sticky	**2.67**	**0.53**
Chloroform	Brownish dark green	Characteristic	Semisolid	Sticky	**4.15**	**0.83**
Methanol	Reddish Brown	Characteristic	Semisolid	Sticky	**7.63**	**1.526**
Ethanol: water (50:50)	Reddish Brown	Characteristic	Semisolid	Sticky	**9.975**	**1.995**

The % yield was maximum (1.995%) obtained with aqueous: ethanol (50:50) and least (0.53%) with petroleum ether media.

Table 2: Phytochemical evaluation of *Ficus religiosa* bark powder extracts

Phytochemical test	Result			
	Petroleum ether	Chloroform	Menthol	Ethanol: water (50:50)
Test for Carbohydrates				
Molisch's test	-	-	+	+
Benedict's test	-	-	+	+
Fehling's test	-	-	+	+
Test for Glycoside				
Legal's Test (test for cardenoloids)	-	-	+	+
Keller killiani's Test (for deoxysugars)	-	-	+	+
Brontrager's Test	-	-	+	+

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Froth test	-	-	+	-
Test for Protein				
Biuret test	-	-	+	+
Test for Amino Acids				
Millon's Test	-	-	-	-
Ninhydrin Test	-	-	-	-
Test for Phytosterol				
Libermann-Burchard Test	-	-	+	+
Libermann-Burchard Test	-	-	+	+
Test for Phenolics and Tannins				
Ferric chloride test	-	+	+	+
Gelatin test	-	+	+	+
Iodine test	-	+	+	+
Nitric acid test	-	+	+	+
Test for Alkaloids:				
Mayer's Reagent	-	-	+	+
Dragendroff's Reagent	-	-	+	+
Hager's Test	-	-	+	+
Wagner's Test	-	-	+	+
Test for Flavonoids				
Shinoda's Test	-	-	+	-
Lead acetate Test	-	-	+	-

Note- (+) positive test, (-) Negative test.

CONCLUSION:

From my study, it is concluded that Phytochemical screening of petroleum ether, chloroform, methanol and Ethanol: Water (50:50) extracts revealed the presence of carbohydrate, glycoside, alkaloid, protein, amino acid, phytosterol, tannin & flavonoids by positive reaction with the respective test reagent. Phytochemical screening showed that maximum presence of phytoconstituents in methanolic and Ethanol: Water (50:50) extracts. Phytochemical screening showed that minimum presence of phytoconstituents in Petroleum ether.

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