Volume 1 Issue 3 2012

ISSN: 2320- 480X

The Journal of Phytopharmacology

(Pharmacognosy and Phytomedicine Research)

Preliminary Phytochemical Analysis of Bark Extract of Ficus Infectoria Plant

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Abstract: The Pakad (***Ficus infectoria***), is a large tree in Moraceae family, traditionally used in treatment of several diseases (diabetes, menstrual disorders, washing ulcers, leucorrhoea, erysipelas, epitaxis antibacterial and antifungal). The present study was carried out to investigate the phytochemical profile of bark of ***Ficus infectoria***. 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. The result of the study could be useful for description and foundation of monograph of the plant.

Keywords: Pakad, Alkaloids, Flavonoids and Phytochemical.

Introduction:

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product. For the quality control of a traditional medicine, the traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment.

Its common name is White Fig. It is locally known as pilkhan. It is a large spreading tree, with occasional aerial root, found throughout the plains and lower hills. They

are also found in Bangladesh, Nepal, Pakistan, Sri Lanka, South west China & Indochina.

Ficus infectoria is a medium sized tree up to 10.0-12.0 m height, and spread in 8.0-10.0m with a no. of branches. The leaves are glossy green, thick, 10.0cm x 6.0cm, alternate narrow and flowers are inconspicuous white. Bark is 5 to 8 mm in thickness and Flat. The colour of Bark and new leaf is brown and reddish pink respectively.[1, 2, 3]

More recent ethnopharmacological studies show that *Ficus infectoria* is used in many parts of the world for the treatment of a number of diseases, e.g. as an antibacterial, antifungal[4] and hyperglycaemic[5] properties in diabetic conditions. Traditionally Decoction's of bark is used for washing ulcers, as a gargle in salivation; also used for menstrual disorders and leucorrhoea. Some of the countries with a long history of traditional medicinal use of guava include India, Bangladesh, Nepal, Pakistan, Sri Lanka, Southwest China, Indochina etc.[6, 7]

Taxonomic Classification: [1, 7]

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Rosales
Family	Moraceae
Genus	*Ficus*
Species	*infectoria*

Figure: Plant *Ficus infectoria* showing leafs, fruits and bark

Materials and method:

Collection of plant materials: The fresh leaves and bark of ***Ficus infectoria*** plant were collected in the month of October from Sultanpur district, Uttar Pradesh. The plant were authenticated by **Dr. Tariq Husain** (Head & Scientist, Biodiversity & Angiosperm Taxonomy), **National Botanical Research Institute** (NBRI), Lucknow. The leaves and bark of *Ficus infectoria* were cleaned, chopped into small pieces and dried under shade at room temperature for seven days .The dried leaves and bark were powdered and stored in air tight containers for the phytochemical investigation.

Preparation of the extract: The bark of *Ficus infectoria* were cleaned and shade dried in open air for 8-10 days then pulverized to dry power using electric grinder. About 80 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:

$$\%\ \text{Extractive yield (w/w)} = \frac{\text{weight of dried extract}}{\text{weight of dried fruit}} \times 100$$

Determination of Extractive value:- The extractive values of dried bark powder of *Ficus infectoria* were determined with different solvents i.e. petroleum ether, chloroform, methanol, ethanol: water (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 constituents.

Preliminary analyses of *Ficus infectoria* bark extract was performed initially to identify various chemical compounds present and to assess physicochemical properties.

The performed preliminary analyses included:

a) Macroscopic evaluation of bark extract:- Macroscopic evaluation of bark extract was performed with respect to colour, odour, taste, touch etc.

b) Analysis of solubility parameters:- Solubility of prepared bark extract of *Ficus infectoria* was determined in various solvents i.e. distilled water, methanol, ethanol, benzene and chloroform.

Preliminary phytochemical screening:- The various extracts of *Ficus infectoria* i.e. petroleum ether, chloroform, methanol and ethanol: water (50:50) were subjected to qualitative chemical analyses to detect the presence of various phytoconstituents.[8, 9]

Test for Carbohydrate:

A small quantity of the extracts was dissolved separately in 5 ml distilled water and filtered. The filtrates were subjected to the following tests to detect the presence of carbohydrates.

Molish′s test:- 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. Formation of violet ring at the junction may indicate the presence of carbohydrates.

Test for reducing sugar:

Fehiling′s test:- Extract filtrates were treated in equal volumes with 1ml Fehling A and 1ml Fehling B solutions, boiled for one minute separately. 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.

Benedict′s test:- Extract filtrates were treated with equal volumes of Benedict′s reagent in test tubes separately. 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.

Test for Glycosides:

Test for cardiac glycosides:

Keller kelliani test (test for deoxysugar):- Barkmixture extract were treated with chloroform and evaporate it to dryness. 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.

Test for Anthraquinone Glycosides:

Borntrager's test:- Barkmixture 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 volumes of dichloromethane. The lower levels of dichloromethan separated and shaken with half its volume with dilute ammonia. A rose pink to red color appeared in the ammonical layer, indicating the presence of glycosides.

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.

Tests for Amino acid and Protein:

Biuret test (General test):- 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. The formation of purplish violet colour may indicate the presence of proteins.

Million′s test (for proteins):- 3 ml test solutions were mixed with 5 ml Million's reagent separately. White precipitate was formed which on heating turned to brick red. It may indicated the presence of amino acids.

Tests for Sterols and Triterpenoids:

Libermann-Burchard test:- Bark extract were treated with few drops of acetic anhydride separately. Boiled and cooled, concentrated sulphuric acid was added from the side of the test tubes. A brown ring at the junction of two layer and the upper layer turning green which indicated the presence of sterols while formation of deep red colour indicated the presence of triterpenoids.

Salkowski's test:- 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 formation of yellow coloured lower layer indicated the presence of triterpenoids.

Tests for tannins and phenolic compounds:

Ferric chloride test:- Small amount of bark extract were shaken with water separately and warmed. 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.

Gelatin test:- 1% gelatin solution containing 10% sodium chloride was added to each bark extract. Formation of precipitate indicated the presence of tannins and phenolic compounds.

Iodine test:- Bark extract were treated with diluted iodine solution separately. Appearance of transient red colour indicated the presence of tannins and phenolic compounds.

Nitric acid test:- Bark extract were treated with dilute nitric acid separately. Formation of reddish to yellowish colour indicated the presence of tannins and phenolic compounds.

Test for alkaloids:

About 500 mg of the bark extract were stirred with about 5 ml of dilute hydrochloric acid separately and filtered. Each filtrate was tested with the following reagents:

Dragendroff's test:- 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.

Mayer′s test:- 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.

Hager′s test:- 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.

Wagner′s test:- 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.

Tests for flavonoids:

Shinoda test (Magnesium Hydrochloride reduction test):- To bark extracts, 5ml. 95% ethanol was added separately. Each mixture was treated with 0.5g magnesium turnings and few drops of conc. HCL. Pink colour, if produced, may confirm the presence of flavonoids.

Alkaline reagent test: - Small quantity of each extract sample was taken and added with lead acetate solution. After few minutes

appearance of yellow colour precipitates which indicated the presence of flavonoids.

Observations:

Table 1: Physical characteristics and % yield of *Ficus infectoria* bark extract

Solvent	Colour of extract	Odour	Consistency	Sense of touch	Amount of extract (gm)	% yield
Petroleum ether	Brownish dark green	Characteristic	Semisolid	Sticky	3.67	5.52
Chloroform	Brownish dark green	Characteristic	Semisolid	Sticky	8.30	12.30
Methanol	Reddish Brown	Characteristic	Semisolid	Sticky	15.27	21.80
Ethanol: water (50:50)	Reddish Brown	Characteristic	Semisolid	Sticky	19.95	27.53

The % yield was maximum (27.53%) obtained with aqueous: ethanol (50:50) and least (5.52%) with petroleum ether media.

Table 2: Extractive value of *Ficus infectoria* bark extract

Solvent	Extractive value
Petroleum ether	5.62 %
Chloroform	6.85 %
Methanol	11.70 %
Ethanol: water (50:50)	20.60 %

The extractive value was found to be maximum (20.60 %) with aqueous: ethanol (50:50) solvent while minimum (5.62%) with petroleum ether.

Table3: Phytochemical evaluation of *Ficus infectoria* leaves and bark (mixture) extracts

Phytochemical Test	Result			
	Petroleum ether	Chloroform	Methanol	Ethanol: Water (50:50)
Test for Carbohydrates				
Molish'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	-	-	+	+
Froth Test	-	+	+	-
Test For Protein				
Biuret Test	-	-	+	+
Test For Amino Acids				
Millon's Test	-	-	-	-
Ninhydrin Test	-	-	-	-
Test for Phytosterol				
Libermann-Burchard Test	-	+	+	+
Salkowski's 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:

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.

Acknowledgement: The authors are grateful to Mr. Suresh Jain, Chancellor, Teerthanker Mahaveer University and Prof. K.K. Jha, Director, College of Pharmacy, Teerthanker Mahaveer University, Moradabad (U.P.) for providing facilities to carrying out research work on *Ficus infectoria* plant.

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