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EVALUATION OF MACROSCOPICAL AND MICROSCOPICAL STUDY, TLC AND HPTLC FINGERPRINTING OF *FICUS RELIGIOSA* LINN. LEAVES

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ABSTRACT: Objective: To rationalize the macroscopical and microscopical study, TLC and HPTLC fingerprinting of *Ficus religiosa* Linn. leaves. **Methods:** The crude ethanolic extract of leaves of *Ficus religiosa* Linn. was using the macroscopical characters powder microscopical study and Preliminary phytochemical investigation of TLC and HPTLC. **Results:** An attempt has been made to highlight this folk herbal medicine through present study which will assist in the identification of fresh as well as dried crude samples of leaves phytochemically, macroscopically, and microscopically. TLC and HPTLC fingerprint profiling were carried out, and the salient qualitative and quantitative parameters are reported. **Conclusion:** The present study will provide referential information for correct identification and help in checking adulteration in the market sample used in the preparation of various herbal medicines. The present observation will also be helpful in the macroscopical and microscopical study, TLC and HPTLC fingerprinting of *Ficus religiosa* Linn. leaves.

Keywords: Macroscopy, Microscopy, TLC, and HPTLC

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INTRODUCTION: *Ficus religiosa* is a variety of fig tree that was already known as the bodhi tree, even before Gautama Buddha sat under its branches meditating and achieved enlightenment. It is the oldest depicted tree in Indian art and literature, and it can be said that this is the mythical 'World Tree' or the 'Tree of Life' of the Indian subcontinent.

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This plant is considered sacred by the followers of Hinduism, Jainism, and Buddhism, and hence the name 'Sacred Fig' was given to it. Siddhartha Gautama is referred to have been sitting underneath a Bo Tree when he was “enlightened” (Bodhi) or "awakened" (Buddha).

Thus, Bo Tree is a well-known symbol of happiness, prosperity, longevity, and good luck [1]. Peepal tree or sacred fig is a large deciduous tree. It is often planted near temples and holy places. It is supposed to be one of the longest- living trees, and there is one in Sri Lanka which is said to be over one thousand years old. This tree grows very fast, and roots are attached to the trunk as if they are pillars supporting it. The tree needs lots of space,

and the soil must be deep enough to let the roots grow down a long way. It is sometimes also called kalpvruksha [2].

MATERIALS AND METHODS:

Plant Material: The plant material was collected from Bundelkhand University Campus Jhansi in January 2015. The plant was identified by local people of that park and authenticated by Dr. Gaurav Nigam (Asst. Professor) Department of Botany, Bundelkhand University, Jhansi (U.P.) India. A herbarium specimen of the plant (BU/Bot./Spe./Pha./11-2016/02) was preserved in the Department of Pharmacognosy of our Institute for further reference.

FIG. 1: TREE OF *FICUS RELIGIOSA* [3]

Macroscopic: Small tree or taller strangling climber, with wide-spreading branches, semi or fully deciduous in monsoon climates and broadly ovate, glossy, leathery, dark green leaves, 5-7 in (12-18 cm) long, with unusual tail-like tips. Bears pairs of rounded, flat-topped green figs, to 1/2 inch (1.5 cm) across, ripening to purple with red dots. The trunk has smooth grey bark, and with age, this trunk becomes irregularly shaped. It becomes leafless for a brief period in dry habitats.

Microscopy: An external features of bark shows that bark differentiated into thick outer periderm and inner secondary phloem. The periderm is differentiated into phellem and phelloderm. Phellem zone is 360 mm thick, wavy, uneven in trans section. Phellem cells are organized into thin tangential membranous layers, and older layers exfoliate in the form of thin membranes, whereas phelloderm zone is broad and distinct and are turned into lignified sclereids. Secondary phloem differentiated into inner narrow non collapsed zone which consists of radial files of sieve tube membranes, axial parenchyma, gelatinous fibers, and outer collapsed phloem consists of dilated rays, crushed, obliterated sieve tube membranes, thick walled and lignified fibers, abundant tannin filled parenchyma cells [8].

Transverse section of bark shows rectangular to cubical, thick-walled cork cells and dead elements of the secondary cortex, consist of masses of stone cells; cork cambium distinct with rows of the newly formed secondary cortex, mostly composed of stone cells towards the periphery. Stone cells found scattered in large groups, rarely isolated; most of the parenchymatous cells of secondary cortex contain numerous starch grains and few prismatic crystals of calcium oxalate; secondary phloem a wide zone, consisting of sieve elements, phloem fibers in singles or groups of two and non lignified; numerous crystal fibers also present; in outer region sieve elements mostly collapsed while in inner region intact; phloem parenchyma mostly thick-walled; stone cells present in single or in small groups similar to those in secondary cortex; a number of ray-cells and phloem parenchyma filled with brown pigments; prismatic crystals of calcium oxalate and starch grains present in a number of parenchymatous cells; medullary rays uni to multiseriate, wider towards outer periphery composed of thick-walled cells with simple pits; in tangential section ray cells circular to oval in shape; cambium when present, consists of 2-4 layers of thin-walled rectangular cells [9].

TABLE 1: ETHNOMEDICINAL USES OF DIFFERENT PARTS

Plant parts	Traditional uses (as/in)
Bark	Astringent, cooling, aphrodisiac, antibacterial against *Staphylococcus aureus* and *Escherichia coli*, gonorrhea, diarrhea, dysentery, hemorrhoids, gastrohelcosis, anti-inflammatory, burn [4]
shoots	Leaves and tender Purgative, wounds, skin diseases [4]
Fruits	Asthma, laxative, digestive [4]
Seeds	Refrigerants, laxative [4]
Latex	Neuralgia, inflammation, haemorrhages [4]
Leaf juice	Asthma, cough, sexual disorders, diarrhea, haematuria, toothache, migraine, eye troubles, gastric problems, scabies [4-6]
Dry fruit	Tuberculosis, fever, paralysis, hemorrhoids [7]

Thin Layer Chromatography (TLC): "Their relative polarities which related to the type and number of functional groups present on a molecule capable of hydrogen bonding."

$$R_f = \frac{\text{Distance traveled by solute front from origin line}}{\text{Distance traveled by solvent front from origin line}}$$

Where, R_f =Retention factor

The ethanolic extract of leaves of *Ficus religiosa* Linn was subjected to Thin Layer Chromatography studies, to find the presence of several compounds which support by the chemical test.

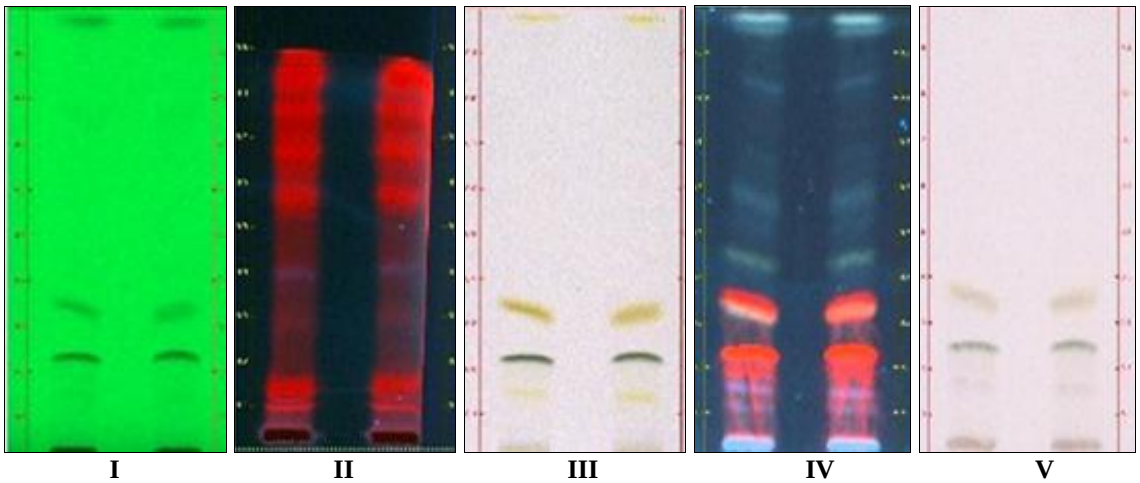
Rf value and color of TLC spots, in a solvent system of Chloroform: Benzene: Formic Acid (5.5:4.5: few drops). These TLC spots with R_f value and color are in **Table 2**, and the TLC plate in **Fig. 2** is given below.

FIG. 2: CHROMATOGRAM OF TLC OF ETHANOLIC EXTRACT OF LEAVES OF *FICUS RELIGIOSA* LINN.

TABLE 2: TLC OF ETHANOLIC EXTRACT LEAVES OF *FICUS RELIGIOSA* LINN.

Extract	Solvent System	No. of Spots	Color of Spots	R_f value
Ethanolic Extract	Chloroform: Benzene: Formic Acid drops) (5.5:4.5: few	8	pink	0.04
			blue	0.10
			Yellow	0.65
			Brick red	0.12
			Orange	0.80
			Green	0.19
			Brown	0.23
			Dark yellow	0.85

HPTLC Profile (High-Performance Thin Layer Chromatography): Ethanolic extract was developed on chromatographic plates with many ratios of different solvents and the best eluent mixture was used further for HPTLC profile to minimize errors in TLC pattern. The preliminary HPTLC studies revealed that the solvent system Chloroform: Benzene: Formic Acid (5.5:4.5: few drops) was ideal and R_f values of HPTLC Fingerprint **Table 7** and **Fig. 7** HPTLC finger-printing ofthe ethanolic extract on leaves of *F. religiosa* given the spots of the chromatogram was visualized at 254 nm and 366 nm.



I **II** **III** **IV** **V**

FIG. 3: HPTLC REPORT OF ETHANOLIC EXTRACT OF LEAVES OF *FICUS RELIGIOSA* LINN.

Where I = 254 nm (before derivatization); II = 366 nm (before derivatization); III = Visible light (before derivatization); IV = 366 nm (after derivatization); V = Visible light (after derivatization)

TABLE 3: R_f VALUES OF HPTLC FINGERPRINT PROFILE OF *FICUS RELIGIOSA* LINN.

R_f Value	Before Derivatization			After Derivatization	
	At 254 nm	At 366 nm	Visible light	At 366 nm	Visible light
R_f 1	0.23 (Black)	0.04 (Pink)	0.14 (yellow)	0.10 (Purple)	0.25 (Green)
R_f 2	0.32 (Black)	0.10 (Blue)	0.23 (Green)	0.14 (Purple)	0.35 (Brownish Yellow)
R_f 3	0.97 (Black)	0.12 (Brick red)	0.32 (Yellowish)	0.23 (Orange)	-
R_f 4	-	0.14 (Blue)	0.98 (Yellow)	0.32 (Orange)	-
R_f 5	-	0.16 (Brick red)	-	0.43 (Faint green)	-
R_f 6	-	0.19 (Orange)	-	0.58 (Light blue)	-
R_f 7	-	0.23 (Orange)	-	0.84 (Light blue)	-
R_f 8	-	0.31 (Orange)	-	0.94 (White)	-
R_f 9	-	-	-	0.98 (White)	-

RESULTS AND DISCUSSION: As a part of the standardization study, the macroscopical examination of the drug was studied. The results showed greater extractive values in hot extraction, indicating the effect of elevated temperature on extraction. The percent extractives in different solvents indicated the quantity and nature of constituents in the extracts. The extractive values are also helpful in estimation of specific constituents soluble in particular solvent. Macroscopic, as well as microscopical studies of any phytodrug are the primary steps to establish its botanical quality control before going to other studies. The above-mentioned parameters are helpful for the future identification and authentification of the plant in the herbal industry and factories. It will serve as a standard data for the quality control of the preparations containing this plant in the future. The leaf constants can be included as microscopical standards in Indian herbal pharmacopeia. Thin layer chromatography (TLC) is particularly valuable for the preliminary separation and determination of plant constituents.

CONCLUSION: The various pharmacological studies can be performed based on phyto-constituents mentioned in the reports. Morphological, microscopical, physicochemical details are helpful for the standardization of the plant.

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CONFLICT OF INTEREST: Nil

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