ORIGINAL ARTICLE

Pharmacognostic Standardization and High-performance Thin-layer Chromatography Fingerprinting of *Ficus infectoria* Leaves

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Abstract

Introduction: *Ficus infectoria* popularly known as a white fig in India and is used in folk medicine to treat various physiological disorders. The pharmacological significance of this traditional Indian medicinal plant has also been justified in the literature by its rich chemical diversity. Due to its rich biochemical properties, plant material was used unauthentically as the traditional medicinal plant product in Indian market. Despite the fact that these plants are sold in the local market by a traditional medicinal healer, we did not find any authenticated data on its quality. **Materials and Methods**: In the present study, quality standards of plant leaves were developed by performing morphological, microscopical, physicochemical, phytochemical, and high-performance thin-layer chromatography (HPTLC) studies using CAMAG Linomat 5 instruments. **Results**: Microscopical studies of leaf and petiole of plant drug showed an arc of centrally located meristele surrounded by lignified parenchymatous tissue. Surface characteristic study of leaf lamina confirms the presence of a paracytic type of stomata. HPTLC data revealed that the primary component in the crude methanolic extract was found at R_f 0.02, 0.12, and 0.52 with the respective peak area of 22.63%, 12.22%, and 13.02%, respectively. **Conclusion**: This study highlighted essential characters which contribute to the standardization, identification, and authentication of plant drugs.

Key words: *Ficus infectoria*, high-performance thin-layer chromatography, microscopy, pharmacognostic study, quality control, standardization

INTRODUCTION

Since ancient times, drugs of natural origin are used for their health-promoting effects and to treat various disorders. Long history of usage and fewer side effects of herbal drugs are making them popular worldwide and alternate choice in health-care system.[1] The Indian traditional system of medicine (Ayurveda, Siddha, and Unani) considers all plant parts as a potential source of the medicinal substance.[2] However, the main obstacle in practice of herbal drugs is the lack of stringent control over the genuineness and adulteration of crude drugs and documentation.[3] This necessitates standardization of the raw herbal drugs. The process of standardization can be achieved by stepwise pharmacognostic studies, namely microscopical, morphological, and physicochemical evaluation.[4]

Medicinal plants form a large group of economically important plants that provide the basic raw materials for indigenous pharmaceuticals. The genus *Ficus* (family Moraceae) comprise more than 800 species of trees.[5] It is of immense medicinal value and constitutes a major part of *Ayurveda* and other indigenous medicine system.[6] One of the prominent members of genus *Ficus* is *Ficus infectoria* Willd. synonym *Ficus lacor* Buch.-Ham (family Moraceae; commonly known as White Fig and Plaksha) which is

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widely distributed in tropical and subtropical regions of the world.[7] It is a large deciduous, rapidly growing tree to a height of about 20 m with the fine shaped crown. The plant is traditionally used in erysipelas, ulcer, epistaxis, leukorrhea, menstrual disorders, and for washing of ulcers. The Ayurvedic Pharmacopoeia of India indicates the use of the fruit and stem bark in syncope, delirium and illusive and unstable state of mind.[7] The leaves are indicated for the treatment of various skin problems. Pharmacological investigations have shown hepatoprotective, antiarthritic, antifilarial, antihyperglycemic, antihyperlipidemia, antimicrobial, and antiulcer activities of *F. infectoria* and thus, validated its ethnopharmacological claims.[8-13] Phytochemical reports showed the presence of long-chain alcohols, methyl ricinolate, terpenoids (alpha- and beta-amyrin, beta-sitosterol, lanosterol, and lupeol), phenols (caffeic acid and bergenin), and flavonoids (sorbifolin and scutellarein derivatives), and coumarin (bergaptol).[7,14]

In spite of tremendous medicinal potential, the quality control standards are not available for *F. infectoria* leaves. Thus, the present investigation was designed to evaluate various pharmacognostical standards including morphology, microscopy, physicochemical parameters, and chemical constituents of *F. infectoria* leaves.

MATERIALS AND METHODS

Plant material

Fresh leaves of *F. infectoria* were collected from the botanical garden of Khalsa College Amritsar, India in the month of March 2017. Taxonomical identification of plant sample was done by senior taxonomist Prof. Parveen Kumar Ahuja, Faculty of Life Sciences, Khalsa University, Amritsar, India. Voucher specimen LSKU/001-18022017 was deposited, and the sample was preserved at the Museum of Khalsa College of Pharmacy, Amritsar, for future reference. The procured sample was rinsed with water and then kept at room temperature until completely dried. The completely dried leaves were used for pharmacognostic standardization.

Morphology, microscopy, and histochemical study

The plant leave samples were evaluated for its color, odor, shape, texture, type, arrangement, and size in the morphological study. Microscopic studies are performed to determine the surface characters, leaf constants, and histochemical characters. Chemo-microscopy studies were carried out to understand the behavior of cells or tissue with different chemicals or stains and help us in determining the nature, type and composition of a cell and their cell wall. Microscopic Transverse section cutting of leaves was done by freehand sectioning method. Fine sections were made cleared with chloral hydrate solution and then stained with the freshly prepared phloroglucinol solution in dilute hydrochloric acid (1:1) and mounted in glycerin.[15] Photomicrographs of microscopic sections were captured with the help of Olympus microscope fitted with the camera using the software.

Physicochemical study

Physicochemical parameters such as moisture content, ash value, alcohol soluble and water-soluble extractive value, and fluorescence analysis were determined by the standard protocol as described in the literature.[16]

Phytochemical study

The dried powders (100 g) of *F. infectoria* leaves were macerated with 80% ethanol (400 ml) for 72 h at room temperature. The filtered crude extract was examined for preliminary phytochemical and thin-layer chromatographic studies as per the standard protocol mentioned in the literature.[16]

High-performance thin-layer chromatography (HPTLC) investigations

HPTLC fingerprinting of a crude hydroalcoholic extract of *F. infectoria* leaves were carried out using CAMAG Linomat 5 instruments equipped with a sample applicator device, Camag twin trough chamber, Camag TLC scanner, and integration software (Wincats). Crude hydro-alcoholic extract was lyophilized and re-dissolved in HPLC grade methanol to yield a drug sample of 15 µl each. Each spot of 2 µl was applied uniformly at a distance of 16 mm on pre-coated silica gel 60 F_{254} plates and plates were then allowed to run in solvent system ethyl acetate:formic acid:acetic acid:water (7:3:1.1:1.1). The plates were scanned at 254 nm for identifying the prominent peaks.

RESULTS AND DISCUSSIONS

Morphology and microscopy

Morphology and microscopy of *F. infectoria* leaves were successfully explored and shown in Figure 1.

It was observed that *F. infectoria* is tree with an average height of 20–25 m. The plant leaves are dark green in color, simple, petiolated, elliptical or oval in shape with acuminate apex and alternate phyllotaxy; 15–18 cm in length and 5–6 cm in width; forked midrib is centrally running through the leaf blade from its base to apex giving off lateral veins in reticulate venation pattern. Surface characteristic of *F. infectoria* leaves was successfully explored and shown in Figure 2.

Plant leaves were observed with rich stomata density, thick forked midrib with reticulate venation and having the paracytic type of stomata.

Transverse section cutting of *F. infectoria* leaves was successfully done, and microscopic characteristic features were determined and shown in Figure 3.

TS of *F. infectoria* leaf passing through midrib is dorsiventral, convex at the bottom side, slightly elevated at the upper side and showed an arc of centrally located broad meristele. Detailed TS of F. infectoria leaf showed upper epidermis consists of a single layer of barrel-shaped cells. The epidermal cells were covered by a thick cuticle embedded with paracytic stomata; underneath the epidermis of midrib lie 2–3 layers of lignified tissue and the remaining tissue being parenchymatous embedded with meristele; consisting of 18–20 rows of radially arranged 2–3 xylem vessels becoming small in size gradually (Plate 3A).

TS of *F. infectoria* petiole was appeared circular. The outermost layer of epidermis covered by cuticle; followed by 3–5 layers of parenchyma cells. The vascular bundles are arranged in a circle, some of the bundles are separated by uni or biseriate parenchyma cells, and each vascular bundle is preceded by fibers. The phloem region is followed by the xylem vessels. The pith consists of wide region of thickened lignified parenchyma cells enriched with starch grains and fibers (Plate 3B).

Quantitative microscopy of plant leaves consists of the determination of leaf constants and its quantitative value displayed in Table 1.

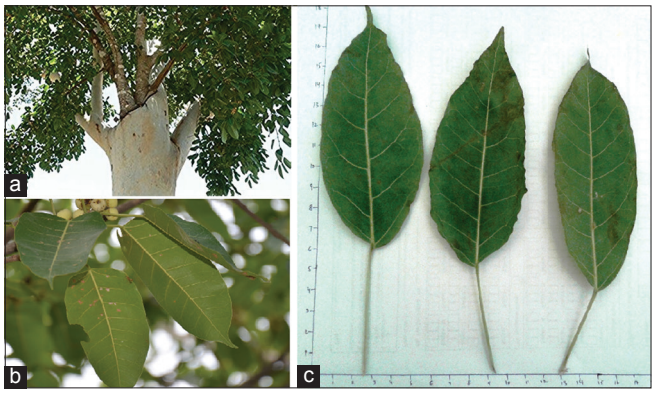


Figure 1: Morphology of *Ficus infectoria* plant (a) entire plant (b) plant part selected for study (c) size determination of plant leaves

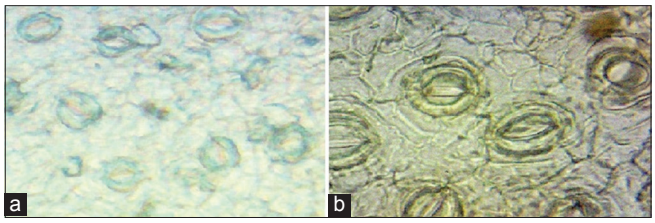


Figure 2: Surface view of *Ficus infectoria* leaves (a) stomata density (b) paracytic stomata

Physicochemical parameters

Different physicochemical parameters were evaluated and were shown in Table 2.

Phytochemical investigations

The preliminary phytochemical analysis of crude hydroalcoholic extracts of *F. infectoria* leaves revealed the presence of most of the polar compounds such as carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, and flavonoids [Table 3].

Table 1: Quantitative microscopy of *F. infectoria* leaves

Parameter	Quantitative value (Range/mm²)
Stomatal index	10±1
Vein islet number	8±1
Vein termination number	11±1

F. infectoria: Ficus infectoria

Table 2: Physiochemical characteristics of plant species

Parameter	*F. infectoria leaves* (%w/w)
Total ash	10.5±0.21
Acid-insoluble ash	0.8±0.12
Water soluble ash	1.5±0.16
Moisture content	11.5±0.18
Alcohol soluble extractive value	9.25±0.17
Water-soluble extractive value	18.28±0.34

F. infectoria: Ficus infectoria

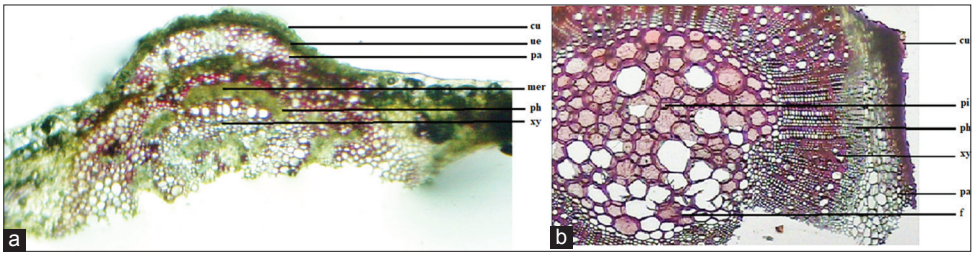
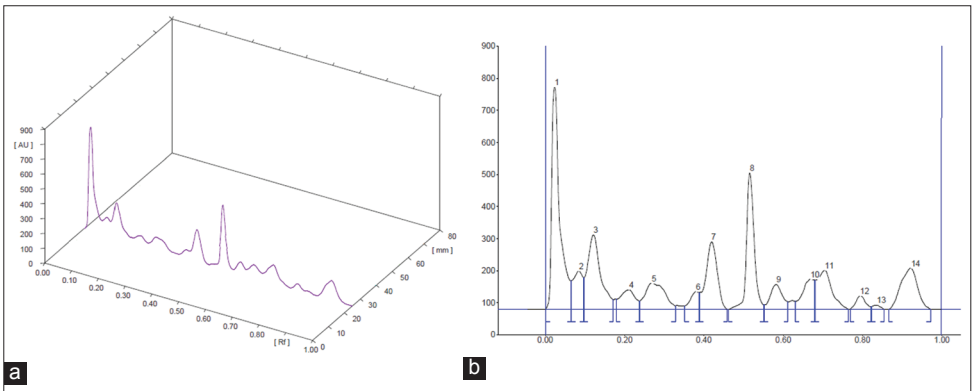


Figure 3: Microscopy of (a) *Ficus infectoria* leaf and (b) *F. infectoria* petiole (cu-cuticle, f-fibers, le-lower epidermis, mer-meristele, pa-parenchyma, ph-phloem, pi-pith, ue-upper epidermis, xy-xylem)

Table 3: Phytochemical screening of FDL and FBL plant extracts

S. No.	Chemical constituent	Tests	*F. infectoria* leaves (Aq-MeOH)
1	Carbohydrates	Molisch's test	+
		Benedict's test	+
		Fehling's test	+
2	Glycosides	Modified Borntrager's test	+++
		Legal test	++
3	Saponins	Froth test	+
4	Phytosterols	Salkowski's test	++
5	Resins	Acetone-water test	-
6	Phenols	Ferric chloride test	++
7	Tannins	Gelatin test	++
8	Flavonoids	Alkaline reagent test	++
		Lead acetate	+

F. infectoria: Ficus infectoria



Peak no.	RF Value	Peak area %
1	0.02	22.63
2	0.09	4.73
3	0.12	12.22
4	0.21	3.62
5	0.27	6.48
6	0.38	1.86
7	0.42	9.56
8	0.52	13.02
9	0.58	3.8
10	0.67	4.46
11	0.71	7.1
12	0.8	1.6
13	0.84	0.39
14	0.92	8.54

Figure 4: High-performance thin-layer chromatography fingerprint of plant leaves extract and their R_f value (a) 2D (b) 1D chromatogram of plant extract

HPTLC Study

The HPTLC chromatographic fingerprint of crude methanolic extracts of *F. infectroia* leaves was developed under the chromatographic conditions described above. Figure 4 showed the HPTLC fingerprinting profile of plant sample, numbers of peaks, their R_f values and percentage area occupied by individual peaks. Three significant peaks at R_f 0.02, 0.12,

and 0.52 were found in high percentage which indicates the high quantity of these corresponding plant constituents. These peaks can be considered as principal components and significant in the identification of *F. infectoria* leaves the sample. The process of isolation and identification of these components are under process and will soon come up with a refined picture.

CONCLUSION

Standardization of plant drug is considered an important factor in the controlling the quality of traditional herbal medicines. Quality standards of *F. infectoria* leaves were developed, and all these parameters could serve as an important lead in the identification of plant drug. The present study attempts to outline basic requirements necessary to develop technical standards for making the medicinal plant worth exploring for further research work.

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