

DEVELOPMENT OF QUALITATIVE PHARMACOGNOSTIC AND HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHIC FINGERPRINTING OF MORPHOLOGICAL SIMILAR SPECIES OF GENUS FICUS

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ABSTRACT

Objective: *Ficus deltoidea* (FD) and *Ficus benjamina* (FB), popularly known as “jack tree/mas cotek” and “pimpri/java fig,” respectively, in India and are used in folk medicine to treat the wound, ulcers, diabetes, microbial infections, and inflammatory disorders. Such therapeutic claims have also been justified in the literature by their rich chemical diversity. Both the plant species are morphologically similar and used unauthentically as the traditional medicinal product. Although these plants are sold in the local market by a traditional medicinal healer, we did not find any authenticated data on its quality.

Methods: In the present study, quality standards of both the plant drugs have been developed and compared by performing morphological, microscopical, physicochemical, phytochemical, and high-performance thin-layer chromatographic (HPTLC) analysis. using CAMAG Linomat 5 instrument (Kindly delete the bold matter).

Results: It was revealed that both the plant species have similar pharmacognostic features with some differences in type of stomata, presence of marked midrib, arrangement of the cell, cell structures, and meristele. HPTLC data revealed that the primary component in FD leaves (FDL) and FB leaves (FBL) extracts was found at R_f 0.67 and 0.37 with the respective peak area of 35.61% and 34.71%. The peaks at R_f 0.17 and 0.27 can be appeared as the chemical marker to highlight the quality of FDL, whereas peaks at R_f 0.87 and 0.95 can be considered to identify and chemically standardize the FBL.

Conclusion: This study highlighted essential characters which contribute to the standardization, identification, and authentication of plant drugs.

Keywords: Ficus, Standardization, High-performance thin-layer chromatographic, Microscopy, Quality control, Pharmacognostic.

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INTRODUCTION

Quality control of medicinal plant can be understood as the unique approach to confirm and document the identity, purity, biochemical quality, and respective quantity of various biochemicals present in raw material by following pharmacognostic, physicochemical, and phytochemical strategies. Development of qualitative and quantitative data of medicinal plants is considered to be of utmost importance because of similarity in phenotypic and chemotypic characters of the different plants either belonging to same or different genus. Genus “Ficus” (Moraceae) comprises of approximately 800 species globally, and of these, 115 species are distributed in India [1]. Several members of this genus were employed as one of the economical sources of medicine because of same or different genus rich chemical diversity [2-5]. The therapeutic and chemical potential of only of its species was extensively explored and reported in the literature. Owing to the morphological, biochemical, and pharmacological similarities among most of the Ficus species, this is understudied genus in modern pharmacognosy. It was also found in the literature that only some morphological, microscopical, and physical parameters were observed which may be considered inappropriate to identify some similar species of ficus [6-9].

Ficus deltoidea (FD) and *F. benjamina* (FB) are two morphologically similar perennial trees; grown up to the height of 4–8 m; widely distributed in the plain area of India, Southeast Asia, Malaysia, and Northern Australia. FD and FB are commonly known as ficus mixed and Benjamin’s fig/Java fig/Pimpri/weeping fig, respectively, in India [10]. The entire plant parts of both the trees were reported as the drug in

traditional medicinal systems. Moreover, the leaves of both the FD and FB were considered in various ethnopharmacological claims. They were reported to have wound healing property, and their decoction was applied on the ulcers with oil.

Further, the ethnopharmacology of FD leaves (FDL) claimed that its decoction is used in the treatment and management of diabetes, menstrual cycle and is also considered as the natural tonic to strengthen the uterine and vaginal muscles after delivery. Preclinical studies on FDL indicated its promising anticancer [11], antidiabetic [12], anti-inflammatory [13], and significant wound healing capacities [14]. The presence of wide range of chemical compounds such as vitexin, isovitexin, rutin, quercetin, and naringenin is also considered as an important factor to accept the ethnopharmacological claims and modern pharmacological studies [15,16]. On the other hand, FB leaves (FBL) were also considered as folklore medicine in the treatment of various skin and respiratory disorders [17]. The leaves and bark of FBL are used indigenously as an insect repellent and in the treatment of malaria. Various authors had successfully documented the scientific reasons for these ethnopharmacological claims by extensively exploring the pharmacological profile and chemical diversity of FBL [18-23].

Despite the extensive use of both these plants in various research institutes for their traditional significance, there is no such report yet available which defines their identity and authenticity. Hence, an attempt has been made to develop the standard pharmacognostic and phytochemical data of the raw form of these plant drugs following the WHO prescribed protocol.

METHODS

Plant material

Fresh leaves of both the plants were collected from the botanical garden of Khalsa College Amritsar, India, in February 2016. Our team members identified specimens, and their taxonomical identification and authentication was done by senior taxonomist Prof. Parveen Kumar Ahuja, Faculty of Life Sciences, Khalsa University, Amritsar, India. Voucher specimen LSKU/001-18022017 was deposited at the Museum of Khalsa College of Pharmacy, Amritsar. The collected plant specimens were washed with water to remove dirt particles from their surface and then kept at room temperature until completely dried. The dried leaves were ground to get a coarse powder.

Morphology, microscopy, and histochemical study

The plants leaf sample were evaluated for its color, odour, shape, texture, type, arrangement, and size in the morphological study. Microscopic studies were 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 [7]. 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 observed by standard methods as described in the literature [24].

Phytochemical study

The dried powders (100 g) of both leaves were macerated with 80% ethanol (400 ml) for 72 h at room temperature. The filtered crude extracts were examined for preliminary phytochemical and thin-layer chromatographic studies as per standard protocol mentioned in the literature [24].

High-performance thin-layer chromatographic (HPTLC) investigations

HPTLC fingerprinting of crude hydroalcoholic extracts of both FDL and FBL was carried out using CAMAG Linomat 5 instrument equipped with a sample applicator device, Camag twin trough chamber, Camag TLC scanner, and integration software (Wincats). Crude hydroalcoholic extracts were lyophilized and redissolved in HPLC grade methanol to yield a drug sample of 15 µl each. Each spot of 2 µl was applied in different tracks at a distance of 16 mm on pre-coated silica gel 60 F₂₅₄ 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 FDL and FBL were successfully explored and shown in Fig. 1.

It was observed that both the plants are morphologically similar and have an average height of 5–7 m. The leaves of both FD and FB are simple, entire, petiolate, dark green, and alternate phyllotaxy. FDL was 3–6 cm in length and 2–3 cm in width and elliptical or oval in shape with mucronate apex; forked midrib was centrally running through the leaf blade from its base to apex giving off lateral veins in reticulate venation pattern. FBL was 5–7 cm in length and 2 cm in width; elliptical or oval in shape with acuminate apex; midrib was not protruding on

leaf blade pinnate reticulate venation.

Surface characteristic of both FDL and FBL was successfully explored and shown in Fig. 2.

It was clearly observed that stomata density was higher in FDL comparative to FBL along with thick forked midrib and reticulate venation in both the plant samples. FDL and FBL showed different types of stomata such as diacytic and paracytic, respectively, which distinguished them from each other.

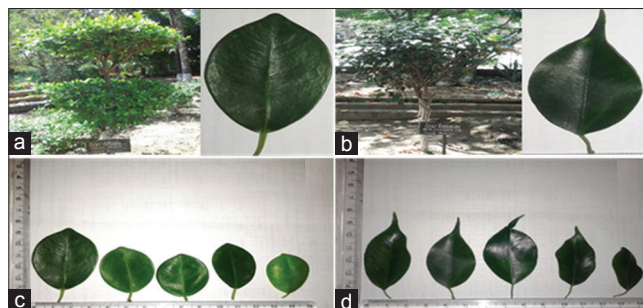


Fig. 1: Morphology of *Ficus deltoidea* leaves (FDL) and *Ficus benjamina* leaves (FBL), (a) entire plant of FD and FDL, (b) entire plant of FB and FBL, (c and d) size determination of FDL and FBL

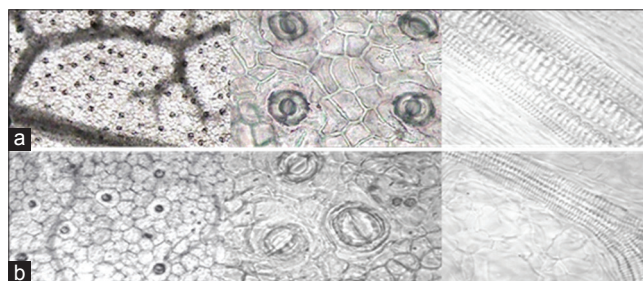


Fig. 2: Surface view of *Ficus deltoidea* leaves (FDL) and *Ficus benjamina* leaves (FBL), (a) reticulate venation, diacytic stomata, and forked midrib in FDL, (b) reticulate venation, paracytic stomata, and centralized midrib in FBL

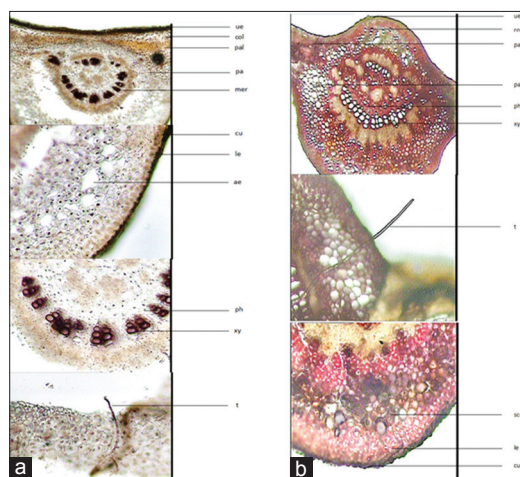


Fig. 3: Microscopy of (a) *Ficus deltoidea* leaves (FDL) and (b) *Ficus benjamina* leaves (FBL) (col - collenchyma, cu - cuticle, le - lower epidermis, mer - meristele, pa - parenchyma, pal - palisade, ph - phloem, scr - sclerenchyma, t - trichomes, ue - upper epidermis, and xy - xylem)

Transverse section cutting of both FDL and FBL was successfully observed and shown in Fig. 3.

T.S. of FDL passing through midrib is dorsiventral in nature, broadly convex on the lower side, slightly centrally depressed on an upper side, and shows an arc of centrally located broad meristeles and rudimentary vascular strands lying above it. Meristeles embedded in parenchymatous ground tissue and consists of 10–14 rows of radially running 2–3 xylem vessels becoming small in size gradually. It showed a layer of upper and lower epidermis covered with cuticle, transverse with a diacytic type of stomata. Underneath, both the epidermis of midrib lie 3–5 layers of collenchymatous cells followed by pitted parenchymatous ground tissue embedded with marked aerenchymatous cells toward lower epidermis. TS of FBL passing through midrib is dorsiventral, convex at the bottom side, slightly elevated at the upper side, and showed an arc of centrally located broad meristeles. Detailed TS of FBL leaf passing through midrib shows a layer of upper and lower epidermis covered with cuticle and embedded with paracytic stomata; underneath, both the epidermis of midrib lie 2–3 layers of lignified collenchymas tissue, the remaining tissue being parenchymatous embedded with meristeles, consisting of 18–20 rows of radially arranged 2–3 xylem vessels becoming small in size gradually.

The measurement of quantitative microscopy data revealed that stomatal density and other leaf constants determined in FDL were higher in range comparative to FBL as indicated in Table 1.

Physicochemical parameters

Different physicochemical parameters were evaluated, and their values are shown in Table 2.

The moisture content was important to determine since an excess of moisture in herbal material promotes the hydrolysis and growth of microbes. The percentage of total moisture content of fresh FDL and FBL was found to be 13.2 and 11.5, respectively. The extractable

Table 1: Quantitative microscopy of plant species

Parameter	Quantitative value (range/mm ²)	
	FDL	FBL
Stomatal index	11±1	9±1
Vein islet number	9±1	6±1
Vein termination number	10±1	7±1

FDL: *Ficus deltoidea* leaves, FBL: *Ficus benjamina* leaves

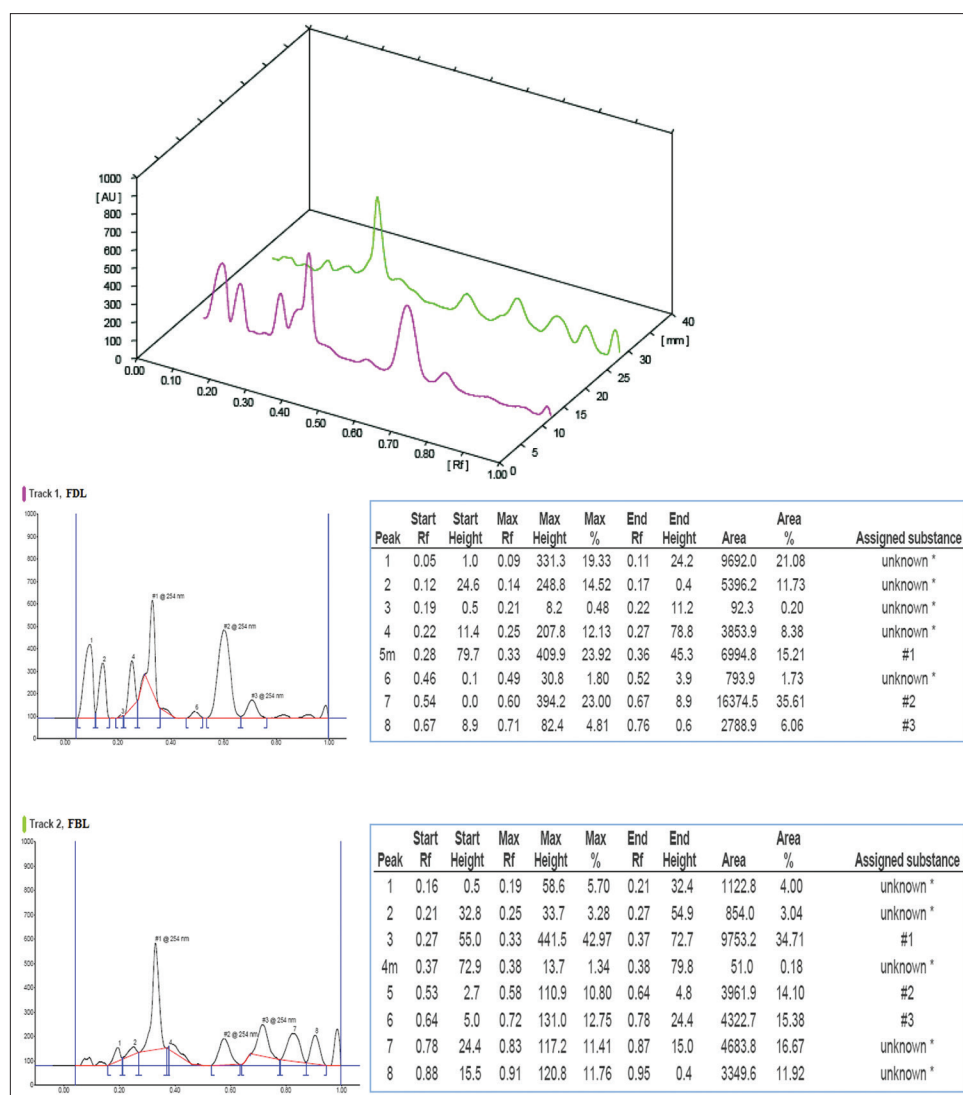


Fig. 4: High-performance thin-layer chromatographic fingerprint of plant leaves extract and their R_f value

matter in plant samples was successfully determined which describes the amount or type of plant constituents extracted with different solvents. Water-soluble and alcohol-soluble extractive values were found comparatively higher in the FDL than FBL which indicates the presence of polar constituents in high quantity in both the plant leaves. The ash value was determined to establish the quality and purity of crude plant materials and to remove all traces of organic matter which may otherwise interfere with an analytical determination. For this, a sample was placed in the muffle furnace for a long period at high temperature so that complete combustion of the sample occurs. The total ash determined in FDL and FBL was 8.2% w/w and 11.7% w/w, respectively, and comparative to total ash values, the high percentage of acid insoluble ash was determined in FBL which indicates the presence of silicate material in relatively higher amount than FDL.

The fluorescence characters of dried powdered plant drugs and acid- or base-treated plant drugs were determined under visible light, short, and long ultraviolet and are presented in Table 3.

These characteristic color properties could be used as a standard for the identification and authentication of the FDL and FBL in its crude form.

Phytochemical investigations

The preliminary phytochemical analysis of crude hydroalcoholic extracts of FDL and FBL revealed the presence of most of the polar compounds

Table 2: Physiochemical characteristics of plant species

Parameter	FDL (%w/w)	FBL (%w/w)
Total ash	8.2±0.21	11.7±0.18
Acid-insoluble ash	0.9±0.14	1.8±0.22
Water-soluble ash	1.7±0.14	2.3±0.08
Moisture content	13.2±0.13	11.5±0.21
Alcohol-soluble extractive value	8.35±0.17	6.72±0.12
Water-soluble extractive value	16.28±0.31	14.20±0.08

FDL: *Ficus deltoidea* leaves, FBL: *Ficus benjamina* leaves

such as carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, and flavonoids (Table 4). The data indicated the presence of all the above-listed chemical classes in FDL extract. However, flavonoids, phenols, and saponins are absent in case of FBL extract. This result indicates that abundant quantity of polar compounds could be present in FDL and FBL.

HPTLC study

The HPTLC chromatographic fingerprinting of crude methanolic extracts of both FDL and FBL was developed under the chromatographic conditions described above. Fig. 4 shows the HPTLC fingerprinting of FDL and FBL, numbers of peaks, their R_f values, and percentage area occupied by individual peaks of both FDL and FBL crude extracts. Three significant peaks at R_f 0.36, 0.67, and 0.76 in both the FDL and FBL extracts were found common. The principal component found in FDL and FBL extracts was found at R_f 0.67 and 0.37 with the respective peak area of 35.61% and 34.71%. The peaks at R_f 0.17 and 0.27 can be considered as unique identity or chemical marker to highlight the quality of FDL, whereas peaks at R_f 0.87 and 0.95 can be considered to identify the FBL and chemical standardization of FB crude drug. The process of isolation and identification of these components is under process and will soon come up with a refined picture.

CONCLUSIONS

Quality standards of FDL and FBL were developed, and significant differences were observed in morphology, microscopy, pharmacognosy, and HPTLC data. Leaves of both the medicinal plants displayed variation in size, shape, and their surface characters, whereas microscopic examination distinguished them by variation in the type of stomata, the presence of marked midrib, the arrangement of the cell, cell structures, and meristele. The presence of aerenchymatous cells in FDL has marked its identity which was utterly absent in FBL. The type, content of organic matter, and the distribution of various components in plant leaves were concluded from physicochemical and fluorescence analysis. Although both FDL and FBL contain almost same phytochemical organic substances, their HPTLC fingerprints showed the presence of some

Table 3: Fluorescence characteristics of ficus species with different reagents

Treatment	Fluorescence characters					
	FDL			FBL		
	Visible light	Short UV (254 nm)	Long UV (366 nm)	Visible light	Short UV (254 nm)	Long UV (366 nm)
Dried Powder as such	Greenish brown	Dark green	Brownish	Greenish brown	Dark green	Brownish
Sodium hydroxide	Dark green	Dark green	Orange brown	Dark green	Dull green	Orange
Hydrochloric acid	Dull green	Green	Dull green	Dull green	Greenish yellow	Brown
Nitric acid	Brown	Dull Green	Dull yellow	Dull brown	Greenish yellow	Brownish yellow
Distilled water	Dark green	Green	Greenish brown	dark green	greenish brown	Brownish yellow

FDL: *Ficus deltoidea* leaves, FBL: *Ficus benjamina* leaves, UV: Ultraviolet

Table 4: Phytochemical screening of FDL and FBL plant extracts

S. No	Chemical constituent	Tests	FDL MeOH	FBL 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	+	+

FDL: *Ficus deltoidea* leaves, FBL: *Ficus benjamina* leaves

distinguishing phytochemicals in them which could be considered as the vital tool for quality analysis, identification, and standardization of individual plant drugs.

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AUTHOR'S CONTRIBUTIONS

All authors have equal contribution.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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