WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 7, 1493-1505. Research Article ISSN 2277– 7105

PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *PHYLLANTHUS FRATERNUS*

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Article Received on
24 April 2020,

Revised on 14 May 2020,
Accepted on 03 June 2020,

DOI: 10.20959/wjpr20207-17776

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ABSTRACT

Phyllanthus fraternus medicinal plant belonging to the family Euphorbiacea is a well-known traditional medicinal plant used in various indigenous systems of medicine. It is widely distributed throughout India. The present study provides pharmacognostical, physicochemical and phytochemical details of the *P. fraternus* useful in laying down standardization and pharmacopoeial parameters.

KEYWORDS: *Phyllanthus fraternus*, pharmacognostic study, phytochemical analysis.

INTRODUCTION

Phyllanthus fraternus Webster Linn [PF] (family, Euphorbiaceae) used as a folklore remedy for the treatment of various diseases of liver by traditional healers and tribals.[1,2] PF is an important plant of traditional system of medicine, which has beneficial therapeutic potential in the management of various diseases.[1,2] PF is reported to posses hepatoprotective, antibacterial, anti-inflammatory, antihyperglycemic and antioxidant activities.[1,2]

Review of literature revealed that only few handful workers studied the structural details of *P. fraternus*.[1,2] Saha and Krishna Murthy studied the structural details of *P. fraternus*.[3] Later Yelene et al., carried out the leaf structural studies.[4] Khatoon et al., studied three species of Phyllanthus.[5] Sharma and Sheela have studied the distinguishing characters of different species of Phyllanthus by using simple microscopic techniques.[6] Sen et al. have studied *P. fraternus* as the source plant of Tamalaki used in the treatment of Tamaka-svasa (Bronchial asthma) and other respiratory disorders by analyzing therapeutic uses, actions, properties, taste, synonyms as well as pharmacognostical characters.[7]

The present study was an attempt to distinguish pharmacognostic features of *P. fraternus*. The species screened for its pharmacognostic features based on their morphologic, microscopic and phytochemical characteristics.[8] The selected extract in-depth analyzed for their HPTLC data including finger print analysis, detection of class of compounds like Lignans, tannins and flavonoids. The study includes highlighting the pharmacognostical characters and HPTLC finger print profile of *P. fraternus* as distinctive features for authentication, identification and standardization purposes.

MATERIAL METHODS

A. Collection and authentication of plant materials: Whole plant of *Phyllanthus fraternus* Webster family- Euphorbiaceae was collected from different parts of Karad taluka [villages like Saidapur and Banawadi] western Maharashtra. The collected species was verified by Botanical survey of India [BSI], Pune. The plant species deposited as a herbarium identified and authenticated by BSI, Pune [Reference No. BSI/WC/Tech./2012/644].

B. Preparation of Hydroethanolic extract of *Phyllanthus fraternus* whole plant (PFHEE): The dried leaves, stems and roots of *P. fraternus* was minced and extracted with 70% ethanol-water in the proportion of 70:30, being stirred and macerated at room temperature (22-28° C) for 15 days. The ethanol evaporated the extract (yield 5-7%) was concentrated to the desired level and stored in a refrigerator.

C. Pharmacognostic studies[9-11]

Morphological characteristics-The plant was examined for shape, size, surface characteristics, texture, color, consistency, odour, taste, etc.

Microscopic characteristics- Free hand sections of root, stem and leaf of P. *fraternus* taken. Sections were cleared with chloral hydrate and then stained with phloroglucinol and hydrochloric acid and mounted with glycerin.

Physicochemical parameters- Physicochemical parameters were determined as per standard guidelines. Total ash value, loss on drying, water-soluble ash, acid insoluble ash, solubility, melting point, pH, analysis, petroleum ether soluble extractive, alcohol soluble extractive value and water-soluble extractive value were determined.

Phytochemical analysis- The qualitative phytochemical tests of crude powder and acetone extract were carried out to identify different phytoconstituents.

HPTLC studies- The experiment was performed on a pre-coated silica gel 60 F-254 (0.2 mm thickness) HPTLC plate (10 x 10 cm, Merck, Germany). Samples were applied on the plate as 7 mm bands, 15 mm apart from the edges of the plate, with a Camag Linomat V sample applicator. The plates were developed to a distance of 80mm at 25 ± 5°C in a Camag twin trough glass chamber. The saturation time was 30min and after development, plates were dried in a hot-air oven, viewed in a Camag UV chamber and the chromatograms were scanned with a Camag TLC Scanner. The Rf values and fingerprint data were recorded using WINCATS software. Initially the solvent system used was toluene: ethyl acetate in varying ratios (2: 1, 85:15 v/v) tried but the plate was not well resolved. Of the various mobile phases tried, toluene: chloroform: ethanol (4:4:1, v/v) gave the best resolution for development of common chromatogram for the analysis of the components of the extracts under study. Well-defined spots obtained when the chamber saturated with the mobile phase for more than 20 minutes, at room temperature. HPTLC fingerprint study demonstrates unique finger print pattern for the similar solvent system. The HPTLC plate pictures were depicted under specific heading of wavelength with the selected solvent system.

RESULTS AND DISCUSSION

1. Morphologic characters- *P. fraternus* is an annual monoecious herb growing up to 60 cm tall, with glabrous, short, hairy, angular, pale brown, vertical shoots; lateral shoots are up to 10 cm long. The photograph of whole plant of *P. fraternus* is depicted in figure 1. The morphologic description of each part of *P. fraternus* is as follows.

Stem: Herbaceous, quite smooth, aerial, erect, green, branching profuse toward the upper region, 30–60 cm in height and up to 4 mm in diameter. Stem naked below with 5–11 pairs leaves bearing branches, pale green, angular, slender, and spreading. The internodes are small, 1–1.5 cm long.

Leaf: The leaf is simple, numerous, somewhat imprecated, alternate, opposite, thin and almost sessile. The upper surface is green and glabrous while the lower surface is pale green and somewhat glaucous in fresh condition,, often in two rows with a whitish rachis, elliptic-oblong shaped, margin entire, apex rounded, obtuse (rarely sub-acute), base rounded, 6–13 by 3–6 mm, unicostate reticulate venations. The main lateral nerves are usually four to five pairs, petioles very short, stipules simple, minute, free-lateral, awl-shaped, lanceolate-subulate, very acute. Taste is slightly bitter and odor indistinct.

Flowers- They are in the axils of leaves. Flowers unisexual, obovate orbicular, stamens 3, filaments fused.

Fruits- They are 3-lobed nearly globosely capsule with 1 mm to 1.5 mm in diameter, smooth and 6-seeded. Seeds are 1 mm long, segmented, yellowish brown, one side with dark brown tubercles, with concentric ridges on the other side.

Root: The taproot is more or less straight, small, 2.5–11.0 cm long, gradually tapering, with a number of whitish fibrous secondary and tertiary roots, external surface light brown, fracture short.

2. Microscopic characters- The observations of the studied microscopic characters are compiled in a comparative manner with respect to three important parts of the plant viz. root, stem and leaf.

a) Root- Transverse section shows four to six layers of cork cells, consisting of thin-walled, rectangular, tangentially elongated and radially arranged cells, filled with reddish-brown content; secondary cortex consists of 8-10 layers of thin-walled, tangentially elongated parenchymatous cells; secondary phloem is a narrow zone consists of sieve tube, companion cells and phloem parenchyma, and the whole is traversed by narrow phloem rays; secondary xylem represented by a broad zone composed of vessels, tracheids, fibers and xylem parenchyma; pith is parenchymatous. The transverse sections showing the microscopic features of root seen in figure 2.

b) Stem- The Transverse section of stem shows epidermal cells are covered with thick cuticle. Single layered sclerenchymatous hypodermis is present just below the epidermis. Cortex divided into three regions: outermost 4–7 layered chlorenchymatous cells, 2–3 layered sclerenchymatous cells and 2–3 layered parenchymatous cells filled with starch grains. Xylem and phloem has usual elements as observed in *P. amarus*. Pith is madeup of parenchymatous, cells large with intercellular spaces. The transverse sections showing the microscopic features of stem of *P. fraternus* is shown in figure 3.

c) Leaf- Transverse section of leaf shows epidermis on either side composed of thin-walled, tangentially elongated cells, covered externally by a thick cuticle; mesophyll differentiated into palisade and spongy parenchyma; palisade single-layered, occupy nearly half the space between the two layers of epidermis. The anisocytic type of stomata is present on both

epidermises. Stomata followed by respiratory cavities beneath; mesophyll layer is composed of three to five layers of loosely arranged cells having a number of veins traversed in this region, a few cluster crystals of calcium oxalate are present in spongy parenchyma. The transverse sections showing the microscopic features of leaf of *P. fraternus* figure 4.

The important cellular characters of the leaf like type of stomata cell wall, margin of lamina, nature of crystals and nature palisade tissue in midrib reported in comparative manner in table 1.

While the other important cellular characters of the leaf like number of stomata, indices of stomata, palisade ratio, vein islet number and vein termination number tabulated in table 2.

3. Powder characteristics - Powder characteristics of *P. fraternus* outlined below.

a) Root: Powder of the root represented abundant fibers, fragments of cork cells, macrosclereids, and simple starch grains.

b) Stem: Powder of the stem represented abundant fibers, cork cells, crystals and few starch grains.

c) Leaf: Powder of the leaves represented epidermal cell, pallisade cells, vascular tissues, crystals and pieces of stomata.

4. Ash values and specific solvent soluble values

The percentage values of total ash, acid-insoluble ash and water-soluble ash are tabulated Table 3. The alcohol-soluble and water-soluble extractives have been tabulated in Table 3.

5. Preliminary Phytochemical screening of standardized *P. fraternus* extracts

The standardized extract of *Phyllanthus fraternus* whole plant (hydro ethanolic extract) was prepared in our lab by maceration technique. The various physical characteristics of the extract used in the present study are outlined in table 4.

The details of the Phytochemical screening tests utilized and their results are summarized in table 5. The reports are available that suggest that the lignans, tannins and flavonoid present in the Phyllanthus species have shown good and high potential biological activities such as hepatoprotective, antioxidant, anti-inflammatory, analgesic, anti-arthritic, anticancer and anti-allergic reactions.[1-5]

6. HPTLC Finger Print analysis of PF extract

Diverse compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks. The TLC procedure was used to develop the mobile phase for the four PF extracts. The extracts were spotted on the TLC plate and different individual solvents as well as a combination of solvents were tried, to get a good separation. For HPTLC analysis through HPTLC techniques, optimization of solvent system was done using combination of solvent system of varying polarity.

Chromatographic finger print analysis of PF extract taken at 254 nm wavelength shows Rf unique loci at 0.26. PFHEE showed presences of 7 compounds. HPTLC finger print profile and 3d spectra of PF extracts taken at 254 nm wavelength recorded in the Figure 5. The details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in table 6. The number of auto generated peaks for PFHEE 8.

Chromatographic finger print analysis of PF extracts taken at 366 nm wavelength shows Rf unique loci at 0.68. PFHEE showed presences of 9 compounds. The number of auto generated peaks for PFHEE 4. HPTLC finger print profile and 3d spectra of PF extracts taken at 366 nm are recorded in the figure 6. The details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in table no 7.

Chromatographic finger print analysis of the derivatised PF extracts taken at 366 nm wavelength shows Rf unique loci at 0.41. PFHEE showed presences of 12 compounds. HPTLC finger print profile and 3d spectra of subsequent derivatization of PF extract taken at 366 nm wavelength is depicted in Figure 7. The number of auto-generated peaks for PFHEE extract are 12. The details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in table 8.

Chromatographic finger print analysis of the PF extracts taken at 540 nm wavelength shows Rf unique loci at 0.68. PFHEE showed presences of 9 compounds. HPTLC finger print profile and 3d spectra of PF extracts taken at 540 nm are recorded in figure 8. While the other details of Rf range, No of Peaks, No of Auto Generated tracks and Maximum height of peaks is summarized in table 9.

Figure 1: Whole plant of *P. fraternus*.

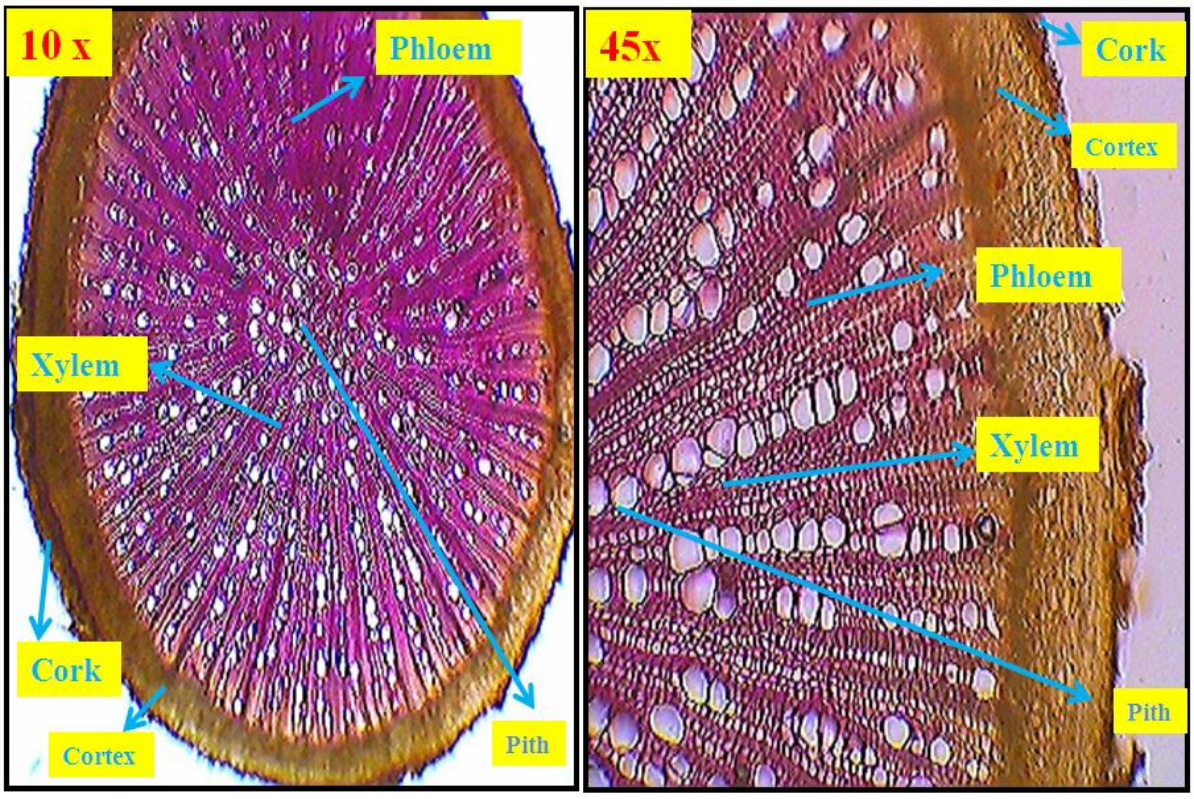

Figure 2: Observed microscopical features of Root of *Phyllanthus fraternus*.

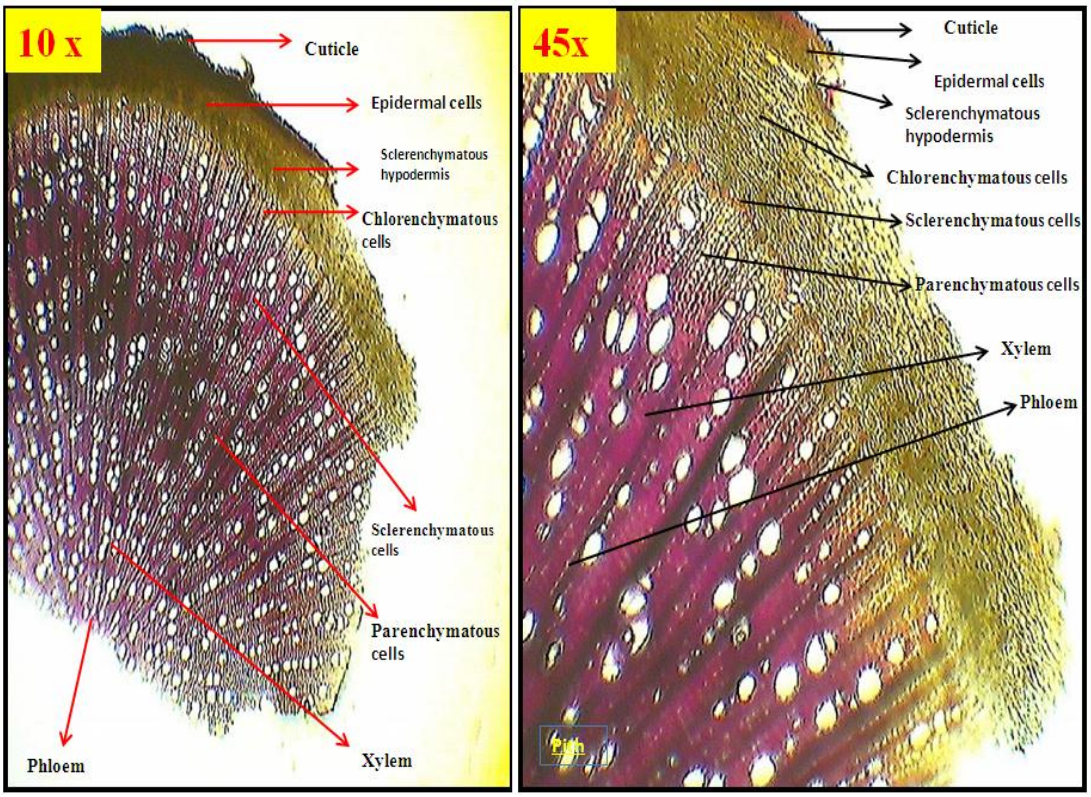

Figure 3: Observed microscopical features of Stem of *P. fraternus*.

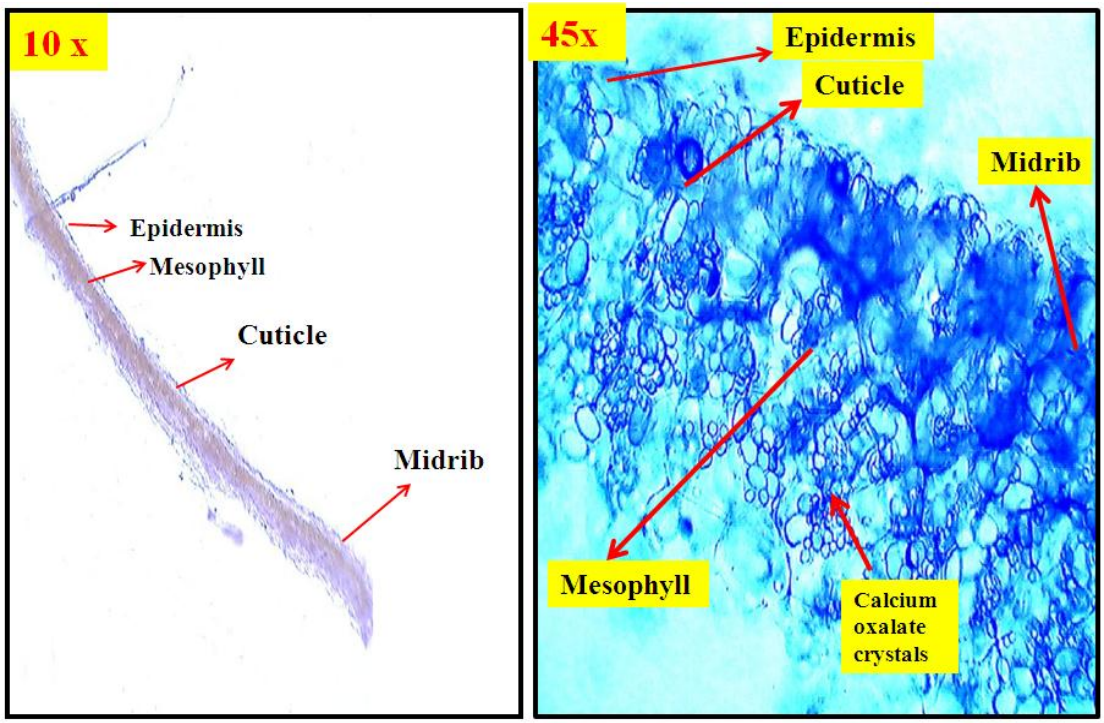

Figure 4: Observed microscopical features of Leaf of *P. fraternus*.

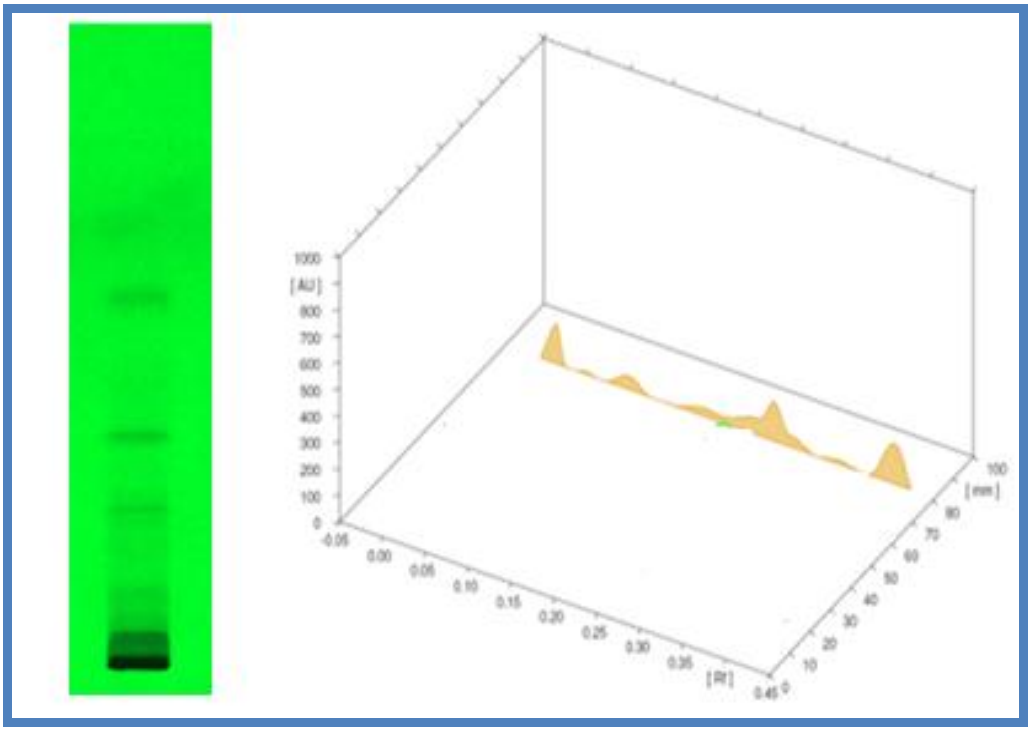

Figure 5: Phytochemical finger print and 3d spectra of PF extracts taken at 254 nm wavelength.

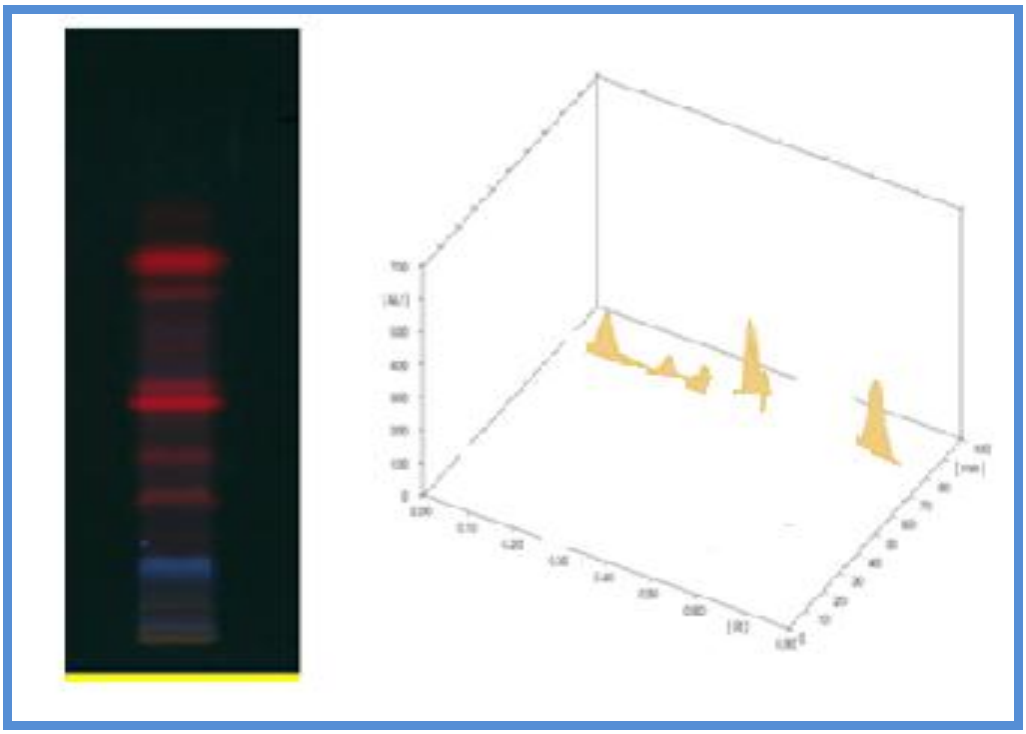

Figure 6: Phytochemical finger print and 3d spectra of PF extracts taken at 366 nm wavelength.

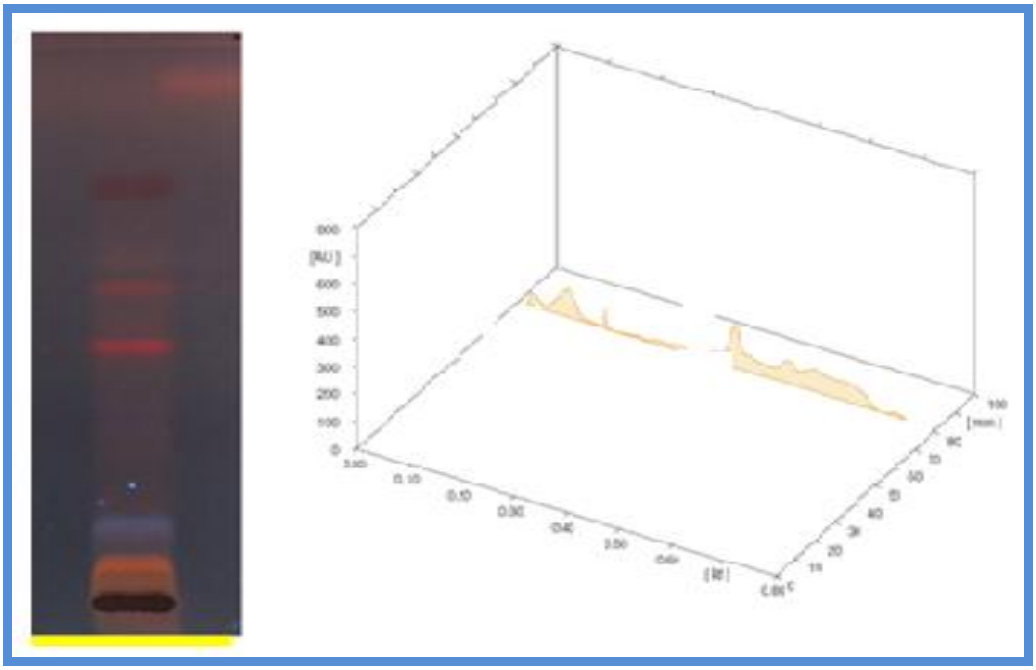

Figure 7: Phytochemical finger print and 3d spectra of derivatised PF extracts taken at 366 nm wavelength

Figure 8: Phytochemical finger print of PF extracts taken at 540 nm wavelength.

Table 1: Important reported comparative cellular characters of the leafs.

Stomatal type	Cell wall	Margin of lamina	Crystals	Palisade tissue in midrib
Anisocyctic	Wavy	Club shaped cells are seen lying in an overlapping manner	Absent	Discontinuous

Table 2: Other important reported comparative cellular characters of the leafs.

Stomatal numbers	Stomatal indices	Palisade ratio	Vein islet number	Vein termination number
186 - 216	20–24	10–11	14–17	31–39

The values are represented as the lower and upper limits of three observations; n = 3.

Table 3: Percentage of ash and extractive values of *Phyllanthus fraternus.*

Parameters	Values in %
Total ash	9.00-10.00
Acid-insoluble ash	0.10 – 0.12
Water-soluble ash	0.14 - 0.18
Alcohol-soluble extract	17.00 - 19.00
Water-soluble extract	24.00 - 26.00

The values are represented as the lower and upper limits of three observations; n = 3.

Table 4: Characterization of extracts by Physical methods.

SR. NO.	TEST	*Phyllanthus fraternus*
		PFHEE
1	**Colour**	Slight brown (when kept under refrigeration) Blackish brown when dried
2	**Odour**	Characteristic
3	**Taste**	Characteristic and agreeable
4	**Solubility**	
	Distilled Water	Good
	DMSO	Excellent

Table 5: Characterization of extracts for various chemical constituents by chemical methods.

CHEMICAL TEST	*Phyllanthus fraternus*
	PFHEE
Test for Flavonoids Shinoda test	+
Test for Tannins Ferric chloride Lead acetate	++
Test for Alkaloids Dragendroff's Test	++
Test for Triterpenoids	+
Test for Sterols Salkowaski Test	+
Test for Reducing sugars (Fehlings test)	+
Test for Saponins Foam Test	+
Test for Glycosides (Molisch test)	+
Test for LIGNANS	++

Interpretation of results: (−) absent; (+) low; (++) good.

Table 6: Chromatographic finger print analysis of PF extracts taken at 254 nm wavelength.

Name of extract	Parameters					
	Rf range	Rf of Unique loci	No of Peaks	No of Auto Generated tracks	Max Height	Area % range
PFHEE	0.01 -0.40	0.26	7	8	10.9 - 152.2	0.89 - 40.25

Table 7: Chromatographic finger print analysis of PF extracts taken at 366 nm wavelength.

Name of extract	Parameters					
	Rf range	Rf of Unique loci	No of Peaks	No of Auto Generated tracks	Max Height	Area % range
PFHEE	0.10 - 0.68	0.41, 0.68	9	4	36.7 -278.3	2.67 - 23.42

Table 8: Chromatographic finger print analysis of derivatised PF extracts taken at 366 nm wavelength.

Name of extract	Parameters					
	Rf range	Rf of Unique loci	No of Peaks	No of Auto Generated tracks	Max Height	Area % range
PFHEE	0.03 - 0.72	0.41	12	4	12.3-23.9	1.02 - 21.86

Table 9: Chromatographic finger print analysis of PF extracts taken at 540 nm wavelength.

Name of extract	Parameters					
	Rf range	Rf of Unique loci	No of Peaks	No of Auto Generated tracks	Max Height	Area % range
PFHEE	0.03 - 0.69	0.52, 0.69	9	4	13.0 - 242.9	0.81 - 31.31

CONCLUSIONS

The present work been taken with a view to lay down standards that could be useful to establish the authenticity of this medicinally useful plant. Macro and micro morphological standards discussed here can consider as identifying parameters to authenticate the drug. In the present study, we have found that most of the biologically active phytochemicals were present in the standardized extract of *P. fraternus*. The medicinal properties of *Phyllanthus fraternus* may be due to the presence of above-mentioned phytochemicals.

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