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Standardization of *Feronia limonia* L. leaves by HPLC, HPTLC, physico-chemical and histological parameters

[Estandarización de hojas de *Feronia limonia* L. por HPLC, HPTLC, parámetros fisicoquímicos e histológicos]

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Abstract
Feronia limonia (Family Rutaceae, subfamily Aurantioideae), commonly known as kaitha or wood apple, is widely used as an ethnomedicine in India. Its leaves are prescribed for a wide variety of ailments like diarrhoea, urinary disorders, treatment of piles or haemorrhoids, acidity, ulcers, ringworm and other chronic skin infections,. However, detailed scientific information is not available to identify the plant material and to ascertain its quality and purity. In the present communication, a qualitative fingerprinting of *Feronia limonia* (FL), extracts have been performed by HPTLC and HPLC methods, which provide qualitative insights into the bioactive constituents present in the extracts. Also, morphological anatomical and physico-chemical characters, along with phytochemical screening and fluorescence analysis of powdered crude drug were carried out for systemic identification and authentification of leaves. This study provides referential information for identification and characterization of FL leaf and its extracts.

Keywords: *Feronia limonia* L.; Leaf; Qualitative phytochemical fingerprinting; Histological parameters; Physico-chemical evaluations; Fluorescence analysis

Resumen
Feronia limonia (Familia Rutaceae, subfamilia Aurantioideae), comúnmente conocida como kaitha o manzana de madera, y es extensamente usada como una ethnomedicina en India. Sus hojas son indicadas para una amplia variedad de dolencias como diarrea, desórdenes urinarios, el tratamiento de hemorroides, acidez, úlceras, tiña y otras infecciones crónicas de la piel. Sin embargo, la información científica detallada no está disponible para identificar el material de la especie y averiguar su calidad y pureza. En la presente comunicación, un análisis cualitativo de extractos de *Feronia limonia* (FL), se realizó por HPTLC y HPLC, lo que proporcionó información cualitativa de los componentes bioactivos presentes en los extractos. También, los caracteres anatómicos, fisico-químicos y morfológicos, junto con un examen fitoquímico y análisis de fluorescencia de la planta en polvo fueron realizados para la identificación sistémica y autentificación de las hojas. Este estudio proporciona la información de referencia para identificación y caracterización de las hojas de FL y sus extractos.

Palabras Clave: *Feronia limonia* L.; Hoja; Huella digital fitoquímica cualitativa; Parámetros histológicos; Evaluaciones fisicoquímicas; Análisis de fluorescencia

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List of abbreviations:
FL-*Feronia limonia* Linn; FL-1-Petroleum ether extract ; FL-2-Toluene extract; FL-3-Chloroform extract; FL-4-Ethylacetate extract; FL-5-Methanol extract; FAA - formalin: acetic acid: 70 % ethyl alcohol; HPLC-High Performance Liquid chromatography; HPTLC-High Performance Thin Layer Chromatograph; PE-Pet ether extract ; TU-Toluene extract; CF-Chloroform extract; EA-Ethyl acetate extract; ME-Methanol extract.

INTRODUCTION

Feronia limonia (Rutaceae, Aurantioideae), is commonly known as 'kaitha' or wood apple (Dreyer *et al.*, 1972) and is widely distributed in deciduous and arid landscapes of several countries in South Asia (Hooker, 1875). *Feronia limonia* (FL) as a whole, or its parts such as unriped fruit, riped fruit, root, bark, trunk gum and leaves have a broad spectrum of traditionally established therapeutic properties (Tiwari and Gupta, 1959) and widely used in several Ayurvedic preparations like *Panch kapittha* (Panda, 2000) and *Kapitthaashtaka churna* (Vayaskara, 1953). The medicinal potential of FL has been known to traditional systems of medicine for a while now with its leaf being widely used. Traditionally, the leaves of FL has been used to treat diarrhoea, urinary disorders, ringworm and other chronic skin infections in *Charak* and *Shushrut Samhita* (Indian Medicinal Treatises) (Khare, 2004). Some ethinic communities of Gujarat and Maharastra (Western India) also consume FL leaves in the treatment of piles or haemorrhoids (Jadeja *et al*., 2006) and in treating acidity/ ulcers (Kamble *et al*., 2010*)*. The leaf extracts of FL has been reported to possess antioxidant (Manjusha *et al*., 2004), larvicidal (Rahuman *et al*., 2000), antidiabetic (Joshi *et al*., 2009) and hepatoprotective (Jain *et al*., 2011; Manjusha *et al*., 2004) activities.

In spite of numerous medicinal uses of FL, standardization parameters for its leaves have not been reported. Hence, the present investigation is an attempt in this direction to evaluate morphological, microscopic and physico-chemical characters along with phytochemical screening and fluorescence analysis of powdered crude drug.

MATERIALS AND METHODS

Plant material

Feronia limonia leaves were collected in September–October 2008 from campus of The M. S. University of Baroda, Vadodara, India. They were authenticated identified and conformed with the herbarium at BARO Herbarium in the Botany Department of The MS University of Baroda, Vadodara and a voucher specimen (Nº Pharmacy/FL / 08-09/01/MJ) has been deposited in the Pharmacy Department, The M. S. University of Baroda, Vadodara, India.

Chemicals and instruments

All the solvents viz. petroleum ether, benzene, chloroform, acetone, ethanol (95%), n-butanol and reagents viz. formalin, acetic acid , ethyl alcohol, tertiary-butyl alcohol, paraffin wax, safranin, fast-green, potassium iodide were of analytical grade and were procured from E. Merck, India. HPLC (Shimadzu, Kyoto, Japan) and HPTLC (Camag, Switzerland) are the major instruments used for the fingerprinting studies. Microscopic photographs were taken using a Magnus MIPS-4613 microscope attached with Magnus live USB 2.0 camera.

Macroscopic and Microscopic analysis

Morphological features of the leaf were analyzed after the collection. For the anatomical studies fresh leaves were collected from the plant and fixed in FAA (formalin: acetic acid: 70% ethyl alcohol). Twenty four hours later, the samples were dehydrated with a graded series of tertiary-butyl alcohol (TBA). Infiltration of the samples was carried out by gradual addition of paraffin wax (melting point 58º – 60º C) until TBA solution attained supersaturation. The sample was then successively embedded into paraffin blocks.

Sectioning

The paraffin-embedded samples were sectioned with the aid of an MC 930 advanced precision rotary microtome. Serial sections were cut at 10 - 12 µm thickness. Dewaxing of the sections was performed by treating the specimen slides sequentially with xylol, xylol + alcohol, alchol, water and finally, with staining fluid (Johansen, 1940). Wherever necessary, sections were also stained with safranin, fast-green and iodine in potassium iodide in order to evaluate starch, stomatal morphology, venation pattern, and trichome presence and distribution. The peels were made by scraping pieces of fresh or softened dried leaves (Glycerine: water mixture) with the help of safety razor blade and stained it with the saffranin and mounted in glycerine. Epidermal peels were also obtained by boiled leaf fragments cut from the mid portion of the lamina and boiled in 10% KOH (Sass,

1964) and stained in safranin after thoroughly washing in distilled water. Glycerin mounted temporary preparations were made for cleared materials. Adaxial and abaxial leaf surfaces from both the species were studied at ×400 magnification and individual cells were identified and measured by micrometer. 20-25 peels were made from each species of several dozen of leaves. All the peels were examined and the representative areas were photographed using Leica research microscope using ×40 objective. The micro powder analysis was done according to the method (Brain and Turner, 1975; Kokate, 1986).

Physico-chemical analysis

Physicochemical analysis i.e., percentage ash values and extractive values were determined according to the official methods described (Indian Pharmacopoeia, 1996) and the WHO guidelines for the quality control methods for medicinal plant materials (WHO, 1992). Fluorescence analysis was carried out according to the method (Chase and Pratt, 1949). For estimation of heavy metals about 5 gm of powdered drug material was ignited in muffle furnace to obtain total ash. 100 mg of the ash was dissolved in 10 ml of 1 N HCl and then the solution was filtered and diluted to 50 ml with distilled water and used for quantitative determination of heavy metals by the atomic absorption spectrophotometer (AAS) (SYSTRONIC 128), coupled with hydride generator and hollow cathode lamps for different elements including heavy metals.

Preliminary phytochemical screening

Preliminary phytochemical tests for the presence of alkaloids, sugars, phenols, flavanoids, saponins, steroids, tannins, coumarins, terpenoids and glycosides were performed on the petroleum ether, chloroform, methanol and aqueous extracts of FL leaves by using standard procedures described by (Kokate, 1986; Harborne, 1998).

HPLC studies

The HPLC analysis of methanolic extract was carried with the chromatographic system (Shimadzu, Kyoto, Japan) consisted of a Shimadzu LC-20 AT Prominence solvent delivery module, a manual Rheodyne injector (Perkin Elmer, Mumbai, India) with a 20 mL fixed loop, and an SPD-20A Prominence (Shimadzu) UV-Vis detector. The separation was performed on a Hypersil C18 column (particle size 5 mm; 250 ´ 4.6 mm id; Thermoquest, Cheshire, UK) preceded by an ODS (Thermoquest) guard column (10 mm, 10 ´ 5 mm id) at an ambient temperature. Chromatographic data were recorded and processed using a Spinchrom Chromatographic Station® CFR Version 2.4.0.193 (Spinchrom Pvt. Ltd, Chennai, India). Peak purity analysis was carried out using an SPD M20A photo-diode array (PDA) detector from Shimadzu. The mobile phase consisted of methanol: water (50:50 v/v) and the separation were performed by using isocratic elution at a flow rate of 0.5 mL min^{-1}. The samples were run for 35 minutes. Detection was done at 254, 366 nm by UV detector.

Sample preparation

FL leaves (5 g coarse powder) was extracted separately twice, with methanol (2 x 45 ml) under reflux (45 min each time) on a water bath. The final extract was taken to dryness in a rotary evaporator and 10 mg of the extract was transferred to a 10 mL volumetric flask and the volume was made up with HPLC grade methanol.

HPTLC fingerprint

Qualitative fingerprinting of successive extracts like pet ether (FL-1), toluene (FL-2), chloroform (FL-3), ethyl acetate (FL-4) and methanol (FL-5) were performed by means of thin layer chromatography (TLC). TLC analyses were carried out on A Camag TLC system equipped with Camag Linomat V an automatic TLC sample spotter, Camag glass twin trough chamber (20 × 10 cm), Camag scanner 3 and integrated win CATS 4 Software were used for the analysis. TLC was performed on a pre-coated silica gel plates (Kieselgel 60F254, Merck, Germany) (Jain et al., 2010), using the mobile phases and spray reagents for detection of secondary metabolite (hexane –chloroform – methanol 1:4.75:0.25; AS reagent). Detection was done under UV at 254, 366 nm and 540 nm. (Wagner et al., 1983).

RESULTS AND DISCUSSION

Macroscopic characters

Feronia limonia is a deciduous erect tree with imparipinnate unipinnate compound leaves arranged in alternate manner. The leaves are leathery and 2.5 - 3 cm long. 3-9 leaflets are petiolate and arranged oppositely on a flat- rachis. They are dark green, obovate with an obtuse notched or cenulate apex. The leaflets are gland dotted and slightly lemon scented when crushed (Figure 1, Table 1).

Figure 1
Photograph of *Feronia limonia* leaves

Table 1
Observations of macroscopic features of *Feronia limonia* leaf

Sr. No.	Features	Observations
1	Colour	Inner surface – Green
		Outer surface – Dark green
2	Odour	Slightly lemon-scented
3	Taste	Bitter
4	Shape	Ovate to obovate
5	Size	Length : 7.5 – 12 cm
		Bridth : 3 – 5 cm

Microscopic characters

Transverse section of leaf – The leaves of FL are dorsiventral or bifacial with distinct adaxial and abaxial faces. In transection the midrib appears to be biconvex with the adaxial surface being more convex than abaxial surface.

A transection through the lamina shows an adaxial/ upper epidermis made up of barrel shaped cells covered with a thick cuticle. The abaxial /lower epidermis is made up of rectangular / barrel shaped cells with thick cuticle which distinctly forms on outer and inner ledges on the guard cells and the subsidiary cells of the stomata. The abaxial epidermis at some location appears multilayered. Below the adaxial epidermis is a single row of large hypodermal cells filled with dense a content that extends into the midrib region.

Mesophyll region is distinctly differentiated into palisade and spongy tissues. Cells of palisade parenchyma are very compactly arranged in four layers, with shorter cells arranged towards the centre of the mesophyll.

The cells of the spongy parenchyma are compactly arranged in strata. The leaves are hypostomatic. Stomata are sunken along with guard cells and restricted to the lower epidermis. A protuberance of the guard cell wall is present above and below the stomatal aperture and appear as horn shaped ledges.

In the midrib region below one/two layers of hypodermis on the adaxial side, collenchyma is present and above the abaxial epidermis two layers of parenchyma are present. Embedded in the isodiametric ground parenchyma, two collateral bundles are arranged oppositely in the form of a closed arc. The vascular bundles are with several xylem elements arranged in radial rows. Phloem lies towards the epidermis. Conducting elements of phloem is seen alternating with sclerenchyma cells. The complete microscopic features of FL leaf are represented in Figure 2 and dimensional details of the leaf are represented in Table 2.

Figure 2
Transverse sections showing various characteristic features of *Feronia limonia* leaf

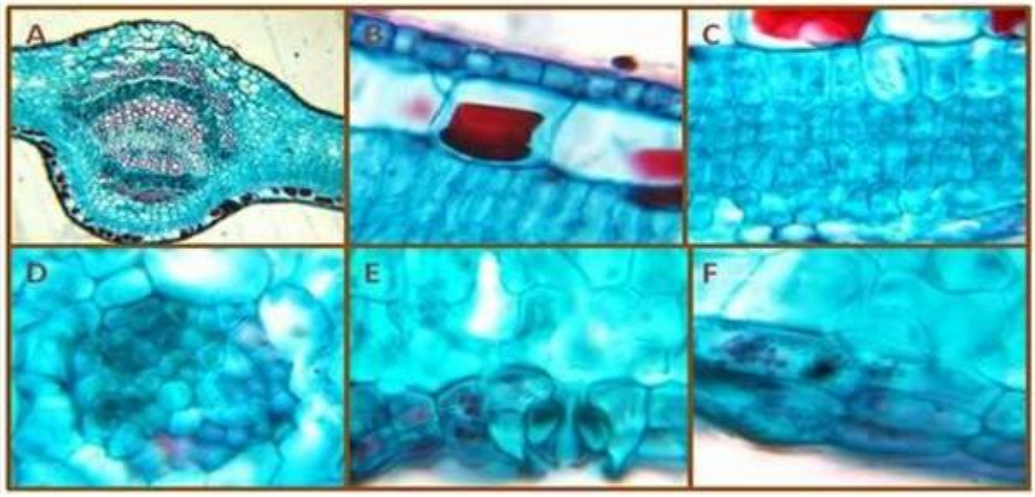


Figure 2 A) Transection through the lamina, B) Adaxial/Upper epidermis with a thick cuticle and hypodermal cells, C) Hypodermal cells with 4 layers of palisade parenchyma, D) Vascular bundle/ lateral veins, E) Stomata with cuteculae ledges, F) Abaxial/lower epidermis.

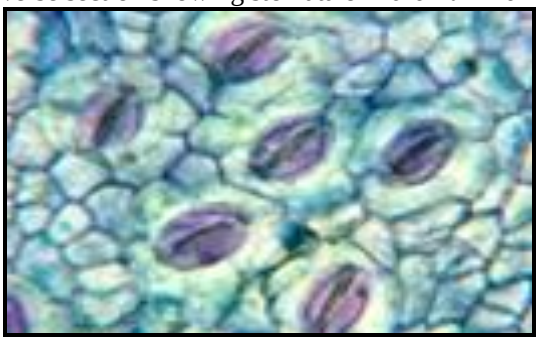
Table 2
Quantitative microscopic features of *Feronia limonia* leaf

Sr. No.	Characteristic features		Size in µm
1	Cuticle Thickness	Lower epidermis	11.10
		Upper epidermis	7.4
2	Upper epidermal layer	Length of cells	14.80
		Width of cells	15.24
3	Lower epidermal layer	Length of cells	14.80
		Width of cells	18.75
4	Hypodermal layer	Length of cells	45.63
		Width of cells	52.17
5	Leaf thickness	Palisade layer	127.85
		Spongy layer	141.71
6	Pallisade layer	Length of cells	35.15
		Width of cells	22.57
7	Spongy layer cells	Length of cells	24.79
		Width of cells	25.9

Surface preparation of leaf/Micromorphological features

Both upper and lower epidermises comprises of polygonal cells with straight or slightly arched anticlinal walls. Trichomes are simple, unicellular and with a very thick wall. Each stoma is surrounded by 4-5 subsidiary cells. The leaves are amphistomatic with anisocytic type of stomata on both surfaces. Stomatal index of the lower epidermis is more than the upper epidermis. The microscopic characteristics examination of the leaf is represented in Figure 3 and quantitative details are represented in Table 3.

Figure 3
Transverse section showing stomata of *Feronia limonia* leaf



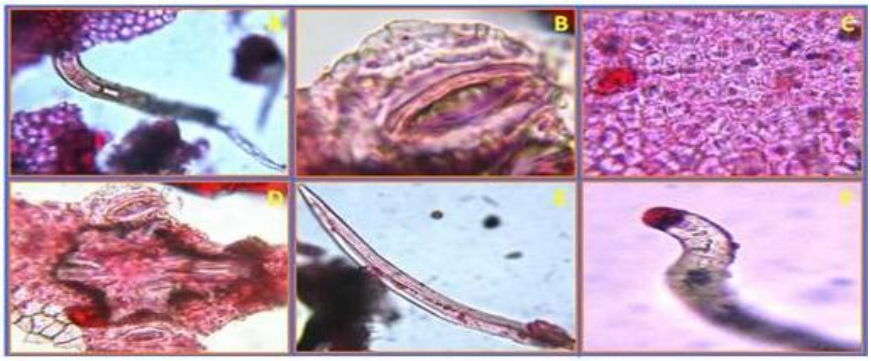
Anisocytic stomata with surrounding subsidiary cells

Table 3
Leaf constants of *Feronia limonia*

S.No.	Leaf constants	Value
1	Stomatal number	
	-Upper epidermis	45
	-Lower epidermis	72
2	Stomatal index	
	-Upper epidermis	0.04-0.05
	-Lower epidermis	5-8
3	Palisade ratio	31.5
4	Vein-islet number	2-3
5	Vein termination number	8-9

Powder characters
The leaf powder is dark green in colour with an unpleasant odour. On microscopical examination, powder of leaves showed the presence of unicellular trichomes, stomata and subsidiary cells of stomata (Figure 4).

Figure 4
Photographs showing various powder microscopic features of *Feronia limonia* leaves



A) Multicellular uniseriate trichome B) Stomata on leaf fragment, C) Polygonal epidermal cells with straight anticlinal walls, D) epidermis with stomata, E) Unicellular trichome with thick cuticle

Physico-chemical studies
Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The percentage of total ash, acid insoluble ash and water soluble ash were carried out. Extractive values are primarily useful for

the determination of exhausted or adulterated drugs. Results of ash value and extractive values are given in Table 4. Percentage yield of successive extracts of various solvents like pet. ether, toluene, chloroform, ethylacetate, methanol and water were also taken into consideration and which were tabulated in Table 5. The colour of the plant extract is mainly due to its chemical composition. The same extract may appear in different colours at different wavelength of light. Kokashi *et al*., (1958) reported that specific colour patterns of various medicinal drugs are obtained at 254 and 366 nm, and are characteristic for a particular drug or different fractions of same drug. In our study, we found a specific colour pattern which is characteristic for leaves of *Feronia limonia*, and hence we claim that it can be used as a finger print for its identification. The results of fluorescence analysis of the methanolic extract of leaf are presented in Figure 5. All the living organisms require inorganic elements for their growth and survival. Medicinal plant contain considerable amounts of mineral constituents (Mg, Mn, Zn etc) is a prerequisite for correct growth and development of plants. Inorganic elements in plants also plays a role in the accumulation of secondary metabolites such as alkaloids, glycosides, terpenoids, phenolic compounds etc. (Andrijany, 1998) as they are responsible for the activity of a number of enzymatic systems, which in turn regulate the metabolic pathways leading to the synthesis of these compounds. The results of inorganic elemental analysis of FL leaves are shown in Table 6.

Table 4

Extractive and Ash values of *Feronia limonia* leaves

Parameters	**Values% (w/w) * ±SEM**
Total ash	10.16±0.33%
Acid insoluble ash	4.13±0.08%
Water soluble ash	0.46±0.03 %
Water soluble extractive value	9.06±0.34 %
Alcohol soluble extractive value	5.43±0.23 %

* Values expressed as mean of three readings; SEM Standard Error Mean

Table 5

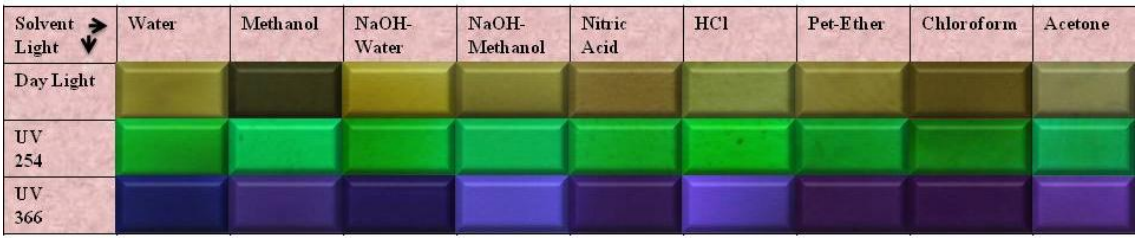
% Yield of successive extract from various solvents by soxhlet apparatus from leaves of *Feronia limonia*

Parameters	**Values% (w/w) * ±SEM**
Pet. Ether extract	1.90±0.05
Toluene extract	0.69±0.02
Chloroform extract	0.95±0.02
Ethyl acetate extract	0.73±0.06
Methanol extract	4.63±0.17
Aqueous extract	9.53±0.17

* values expressed as mean of three readings; SEM Standard Error Mean

Figure 5

Fluorescence analysis of the methanolic extract of *Feronia limonia* leaves at day light, 254 and 366 nm



Colour pattern of *Feronia limonia* leaves methanolic extract under ordinary light and UV- 254 and 366 nm with different solvents

Table 6
Inorganic elemental analysis of *Feronia limonia* leaves

Elements	Values in ppm
Potassium	4215.6
Sodium	279.18
Iron	342.85
Magnesium	8.6
Zinc	29.05
Copper	8.0
Mangnese	11.34

Preliminary phytochemical screening

Preliminary phytochemical screening revealed that flavanoids, coumarins and terpenoids are present in all successive extracts. Along with this, pet ether extract shows the presence of terpenoids whereas, chloroform extract shows the presence of phenols and terpenoids. Similarly, methanolic extract gave a positive test for the presence of phenols, saponins and tannins whereas, aqueous extract showed the presence of phenols and glycosides.

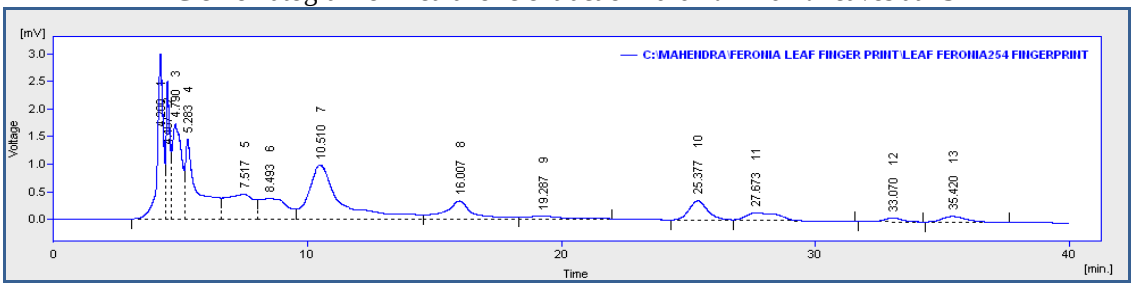
HPLC studies

Standardization and characterization of herbal drugs is a topic of continuous scientific interest in the herbal drug industry. With the advent of modern chromatographic systems there is an ever increasing intent to produce and develop easy, rapid, convenient and cost effective methods for standardization (Selvamani *et al*., 2009). For standardization of methanolic extract of FL leaves, HPLC is a sensitive and accurate tool that fulfils the above mentioned requirements and is widely used tool for the quality assessment of plant extract and its derived product/formulation.

Results of HPLC analysis of FL methanolic extract, at 254 nm, shows presence of various constituents as evidenced by the chromatogram obtained at various retention times (4.200, 4.467, 4.790, 5.283, 7.517, 8.493, 10.510, 16.007, 19.287, 25.377, 27.673, 33.070 and 35.420 min). Similarly, at 366 nm the FL methanolic extract showed various peaks at 4.217, 4.617, 7.550, 8.563 and 15.983 mins.

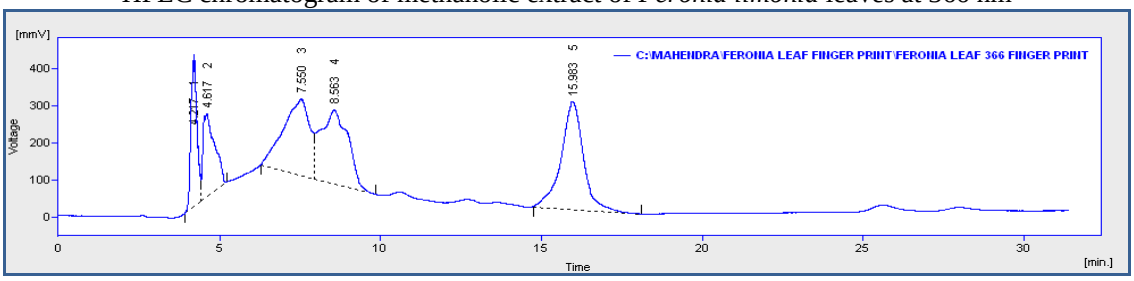
Based upon the fingerprints, it can be concluded that this analytical technique is a convenient method to identify the presence of numerous constituents present in the methanolic extract of FL leaves.

Figure 6
HPLC chromatogram of methanolic extract of *Feronia limonia* leaves at 254 nm



HPLC analysis of leaves methanolic extract (mobile phase, Acetonitrile-water; 75: 25, flow rate; 1ml/min, detection; UV at 254 nm)

Figure 7
HPLC chromatogram of methanolic extract of *Feronia limonia* leaves at 366 nm



HPLC analysis of leaves methanolic extract (mobile phase, Acetonitrile-water; 75: 25, flow rate; 1ml/min, detection; UV at 254 nm)

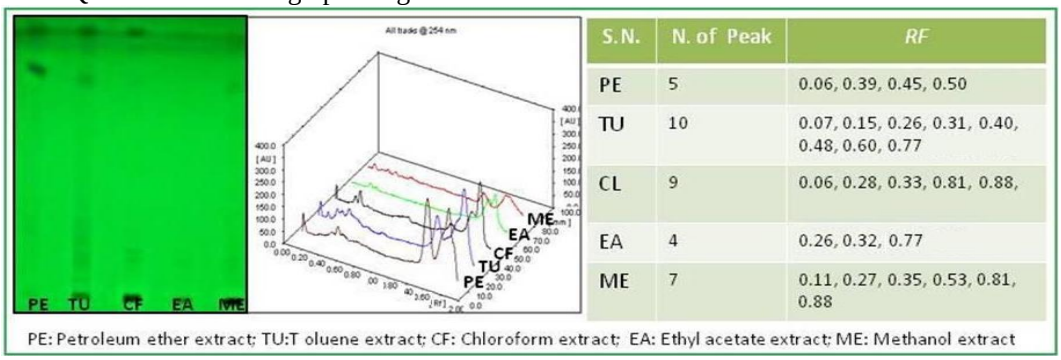
HPTLC fingerprint

The advantage of TLC is the high sample throughput which results from the small amount of sample preparation coupled with simultaneous quantification of several samples. For the separation and quantification of secondary metabolites from botanical materials, high performance thin-layer chromatography has emerged as one of the most efficient tool in the last two decades.

Additionally, numerous samples can be run in a single analysis thereby, dramatically reducing analytical time. With HPTLC, the same analysis can be viewed using different wavelengths of light and thus providing a more complete profile of the plant as compared to other conventional modes of analyses (Dhalwal *et al*., 2008; Singh *et al*., 2005).

In the present study qualitative analysis of secondary metabolites present in successive extracts of leaves FL, was carried out using TLC (silica gel aluminium plate 60F-254, 0.5 mm with hexane/ chloroform/methanol 1:4.75:0.25). Phytochemical analysis provided qualitative insights into the constituents of the FL-1, FL-2, FL-3, FL-4 and FL-5. TLC characterizations of all the successive extracts, showed many spots with different R_f values. Detection was done at UV 254, 366 and 540 nm for resolving metabolites like flavanoids, coumarins, polyphenols etc. The documentation of TLC is shown in figure 8, 9 and 10.

Figure 8
Qualitative TLC fingerprinting of successive extracts from FL leaves at UV-254nm

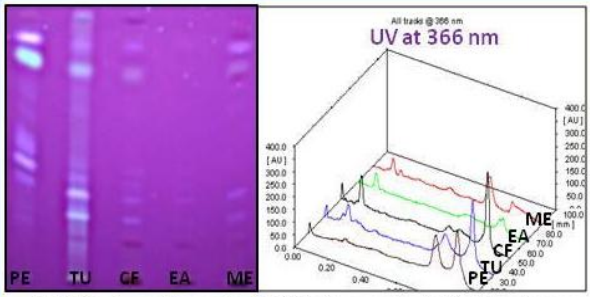


S.N.	N. of Peak	RF
PE	5	0.06, 0.39, 0.45, 0.50
TU	10	0.07, 0.15, 0.26, 0.31, 0.40, 0.48, 0.60, 0.77
CL	9	0.06, 0.28, 0.33, 0.81, 0.88,
EA	4	0.26, 0.32, 0.77
ME	7	0.11, 0.27, 0.35, 0.53, 0.81, 0.88

PE: Petroleum ether extract; TU:T oluene extract; CF: Chloroform extract; EA: Ethyl acetate extract; ME: Methanol extract

TLC profile, three dimensional overlaid spectrum and qualitative details of chemical constituents present in successive extract of *Feronia limonia* leaves at 254 nm

Figure 9
Qualitative TLC fingerprinting of successive extracts from FL leaves at UV-366 nm

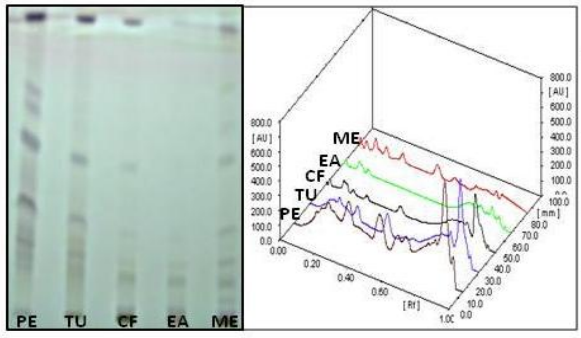


S.N.	N. of Peak	RF
PE	4	0.01, 0.22, 0.76, 0.88
TU	7	0.01, 0.11, 0.14,0.21, 0.30, 0.71, 0.88
CL	5	0.01, 0.12, 0.64, 0.71, 0.87
EA	5	0.02, 0.11, 0.67, 0.84, 0.87
ME	5	0.12, 0.16, 0.46, 0.69, 0.83

PE: Petroleum ether extract; TU:T oluene extract; CF: Chloroform extract; EA: Ethyl acetate extract; ME: Methanol extract

TLC profile, three dimensional overlaid spectrum and qualitative details of chemical constituents present in successive extract of *Feronia limonia* leaves at 366 nm

Figure 10
Qualitative TLC fingerprinting of successive extracts from FL leaves at UV-540 nm



S.N.	N. of Peak	RF
PE	13	0.14, 0.16, 0.20, 0.23, 0.32, 0.38, 0.51, 0.61, 0.66, 0.80, 0.84, 0.90, 0.94
TU	11	0.12, 0.14, 0.17, 0.28, 0.46, 0.56, 0.62, 0.78, 0.83, 0.89, 0.95
CL	10	0.02, 0.11, 0.16, 0.24, 0.44, 0.77, 0.80, 0.83, 0.88, 0.96
EA	10	0.02, 0.10, 0.15, 0.76, 0.80, 0.82, 0.85, 0.89, 0.93, 0.96
ME	12	0.01, 0.05, 0.10, 0.17, 0.26, 0.47, 0.57, 0.63, 0.70, 0.76, 0.82, 0.85

PE: Petroleum ether extract; TU:T oluene extract; CF: Chloroform extract; EA: Ethyl acetate extract; ME: Methanol extract

TLC profile, three dimensional overlaid spectrum and qualitative details of chemical constituents present in successive extract of *Feronia limonia* leaves at 540 nm after derivatization with AS

CONCLUSIONS

The present study of HPLC, HPTLC fingerprinting on the extracts from leaves of *Feronia limonia* will provide useful information for its identification. Morphological, microscopic and physicochemical standards discussed herein can be considered as identifying parameters to substantiate and authenticate the drug.

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