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Hptlc Fingerprint Profiling of Hydroalcoholic Extracts from *Ficus Carica* and *Morus Alba*: A Comparative Phytochemical Analysis

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KEYWORDS

HPTLC fingerprint profiling, Hydroalcoholic extract, *Ficus carica (fig), Morus alba (white mulberry)*, Phytochemical analysis, Biomarker identification, Rutin

ABSTRACT:

Introduction: High-performance thin-layer chromatography (HPTLC) is a robust analytical technique for phytochemical fingerprinting and standardization of herbal extracts. However, comparative fingerprint profiling of *Ficus carica* (fig) and *Morus alba* (white mulberry) hydroalcoholic extracts has not yet been explored

Objectives: This study aims to develop and validate HPTLC methods to generate and compare chemical fingerprints of hydroalcoholic extracts of *F. carica* and *M. alba*.

Methods: Leaves and bark of authenticated *F. carica* and *M. alba* were shade-dried, powdered, and extracted with 10% ethanol and 1% water via Soxhlet. Preliminary phytochemical screening was performed to detect major metabolite classes. HPTLC separation used silica gel 60 F_{254} plates with an optimized mobile phase Toluene: Ethyl acetate: Formic Acid (6: 4: 0.2). Samples were applied as 6 mm bands, developed to 8 cm in a saturated chamber, then visualized under UV 254/366 nm and post-derivatized with anisaldehyde-sulfuric acid. Densitometric scanning was conducted at 366 nm.

Results: Both extracts exhibited rich phytochemical profiles including flavonoids, phenolic acids, tannins, and alkaloids with different Rf valve.

Conclusions: At 254 nm, many conjugated phytochemicals—especially alkaloids, phenolics, flavonoids—absorb strongly, making their spots appear as *dark bands* on plates coated with F_{254} silica gel.in the HPTLC analysis of sample there was many unknow compound found in the sample

Introduction Traditional herbal medicines rely on complex mixtures of phytochemicals, making standardization a critical step for ensuring safety, efficacy, and consistency. High-performance thin-layer chromatography (HPTLC) has emerged as a versatile tool in herbal analysis, offering robust fingerprinting capabilities for the identification, differentiation, and quality control of botanical extracts[1].

Ficus carica (fig) and *Morus alba* (white mulberry) are globally recognized medicinal plants, valued for their polyphenols, flavonoids, tannins, and bioactive phenolic acids. Numerous HPTLC studies have profiled individual extracts—*F. carica* for flavonoids like quercetin [2].and *M. alba* for rutin, chlorogenic acid, and other antioxidants [3]. These analyses underscore HPTLC's ability to both identify key markers and quantify them for quality assurance

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Yet, no study to date has performed a **direct comparative HPTLC fingerprint analysis** of hydroalcoholic extracts of *F. carica* and *M. alba*. Such comparative profiling offers insight into shared phytochemical signatures—useful for authentication—and species-specific markers that enable differentiation. Generating comprehensive HPTLC fingerprints for both plants not only strengthens quality control protocols, but may also support IP, regulatory compliance, and the development of reference materials.

Ficus carica (Common Fig)

Ficus carica L., commonly known as the fig and belonging to the Moraceae family, has been used in traditional medicine across cultures for centuries[4]. Its leaves, fruits, latex, and root bark are known for diverse therapeutic applications: laxative, expectorant, anti-inflammatory, antispasmodic, hepatoprotective, and wound-healing uses[5]. Nutritionally rich in vitamins, minerals, dietary fiber, and a broad spectrum of polyphenols—including flavonoids, furanocoumarins like psoralen, anthocyanins, and phytosterols—*F. carica* also exhibits potent antioxidant activity[6]. Modern studies further support its use in treating gastrointestinal, respiratory, cardiovascular, and inflammatory disorders, with extracts showing efficacy in mitigating oxidative stress and phytophotodermatitis risk linked to furanocoumarins in leaf sap [7].

Morus alba (White Mulberry)

Morus alba L.—commonly referred to as white mulberry—is a fast-growing tree widely cultivated for sericulture, human consumption, and fodder. In Asia, it has longstanding use in traditional medicine: antihypertensive, antidiabetic, antipyretic, anti-inflammatory, antimicrobial, and diuretic applications are common[6]. Mulberry leaves and fruits are rich sources of polyphenols, phenolic acids (e.g., chlorogenic acid), flavonoids (such as rutin, quercetin glycosides, and anthocyanins), tannins, alkaloids, and steroids Morales[8]. Pharmacological studies have demonstrated antioxidant, hypoglycemic, hypolipidemic, neuroprotective, and cardiovascular benefits. Notably, *M. alba* is included in Chinese and Ayurvedic pharmacopeias, reinforcing its medicinal relevance across diverse geographic traditions[9].

Objectives

The main objectives of HPTLC fingerprint profiling of hydroalcoholic extracts from *Ficus carica* and *Morus alba* are to establish a comprehensive comparative phytochemical profile of both plants and to provide a reliable tool for their quality assessment. The study aims to identify and differentiate the characteristic chemical constituents present in the bark extracts of these medicinally important species using hydroalcoholic solvents, thereby generating distinct chromatographic fingerprints. Such profiling not only helps in confirming the authenticity and purity of the plant materials but also aids in detecting adulteration and ensuring standardization for their use in pharmaceutical formulations. Additionally, the objective is to highlight similarities and differences in the phytochemical composition of *F. carica* and *M. alba*, which may correlate with their therapeutic properties. Ultimately, this work is intended to contribute towards the development of a scientific basis for quality control, pharmacological validation, and future research on their bioactive components.

Materials and Methods

Chemicals

Ethanol, hexane, Toluene, Ethyl acetate, Formic Acid were bought from Renkem by avatar performance materials India. HPTLC instrument were obtained from CAMAG Linomat 5 "Linomat5_192428" S/N 192428 (1.00.13)

Collection and authentication of the plant

The fresh barks of *Ficus carica* was collected from Kukrail picnic spot, Lucknow, Uttar Pradesh, India. The bark of *Ficus carica* plant was authenticated by the Prof. N. K. Pandey, Centre of Advanced Study in Botany, Institute of Sciences, Banaras Hindu

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University, Varanasi- 221005, UP, India (plant voucher no. mora. 2023/1).

Extraction process

The fresh barks of *Ficus carica* were cleaned, washed, and dried under shade. These were grinded into a fine powder for optimum extraction. It was extracted through Soxhlet apparatus using hydroalcoholic (ethanol 10%: distilled water 1%) solution for 48 hours. After the extraction process, it was filtered with filter paper and finally with Whatman filter paper. The filtrate (slurry) was made concentrated using the rotatory evaporator. Thus, the herbal extract obtained in dried powdered form and stored in to desiccator to prevent the *Ficus carica* extract from the moisture[11].

S.No.	Sample code	Name of Sample
1	F1	**MLE** (*Morus alba* leave extract)
2	F2	**MBE** (*Morus alba* bark extract
3	F3	**FCB** (*Ficus carica* bark extract)
4	F4	**FCB**-soluble ethyl acetate extract
5	F5	**FCB**-insoluble ethyl acetate extract)

HPTLC finger printing [at 254nm]

Pre-coated silica gel 60 F_{254} aluminium plates were used in stationary phase. Mobile phase includes Toluene: Ethyl acetate: Formic Acid (6: 4: 0.2). The chamber saturation time was kept for 20 mins. In test solution, 1g of dried formulation dissolved in methanol and then filtered the liquid extract. Volume was made up to 10ml using methanol. Visualization and detection was performed at wavelengths i.e., 254 nm, 366 nm and 510 nm[12].

HPTLC Profile-

Conditions

1. Stationary phase: Pre-coated silica gel 60 F_{254} aluminium plates
2. Mobile phase: Toluene: Ethyl acetate : Formic Acid (6 : 4 : 0.2)
3. Chamber Saturation Time: 20 mins.
4. Test Solution: 1 gm of dried formulation dissolved in methanol and then filter the liquid extract. Make the volume up to 10 ml with methanol.
5. Visualization & Detection: 254 nm, 366 nm and 510nm.

Procedure

1. Take previously washed with methanol and dried TLC plate and fix dimension at X position and mark from base with help of pencil at 10 mm and 90 mm. and also left 15 mm from both sides of plate.
2. Apply the test sample solution 10, 15 and 20 µl in the form of bands with the programming of Linomats applicator.
3. Allow the solvent to be evaporated and place the plate in the saturated tank, possibly vertical and so that spots or bands are above the level of mobile phase. Close the tank and allow standing at room temperature until mobile phase ascended to the marked line.
4. Remove the plate and dry and visualize as in UV-Vis light at 254 nm and 366 nm.
5. Prepare the scanning programme for completely dried plate at wavelength of 254 nm and another program to scan at 366 nm.
6. After development, allow the plate to dry in air. Spray the plate with 5% sulphuric acid in methanol reagent followed by heating at 105 deg. cel. for about 10 min. Again scan at 510 nm.
7. Procure the report file containing the images and graphical data of the scanning chromatograms.

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Results

HPTLC finger printing [at 254nm]

Table 1. HPTLC finger printing [at 254nm]

S. N.	F1		F2	
	R_f Value	Area	R_f Value	Area
1	0.11	2593.8	-	-
2	0.16	2015.7	0.29	38823.1
3	0.12	3127.3	0.38	19073.6
4	0.28	1433.4	0.42	16628.1
5	0.37	3527.4	0.47	13732.6
6	0.42	1741.7	0.49	14255.7
7	0.48	2870.2	0.55	24871.2
8	0.51	3481.7	0.71	76234.6
9	0.56	5087.9	0.84	13179.6
10	0.91	299.7	0.93	3970.3

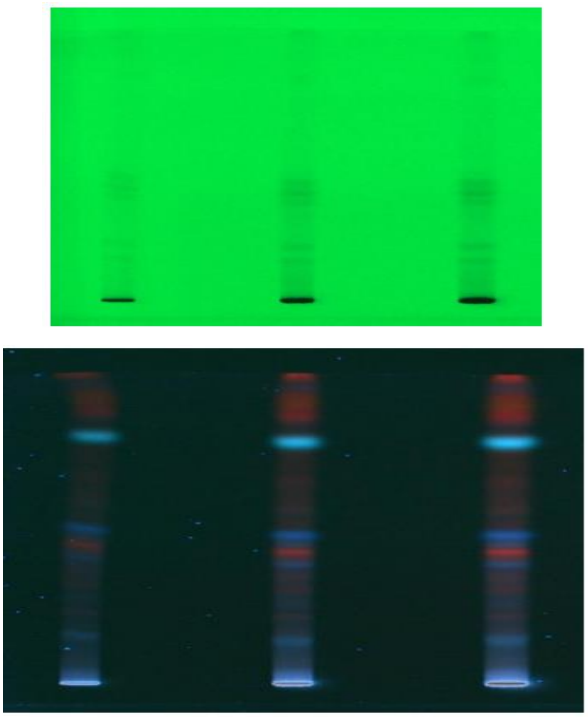

Fig 1. Chromatogram of F1

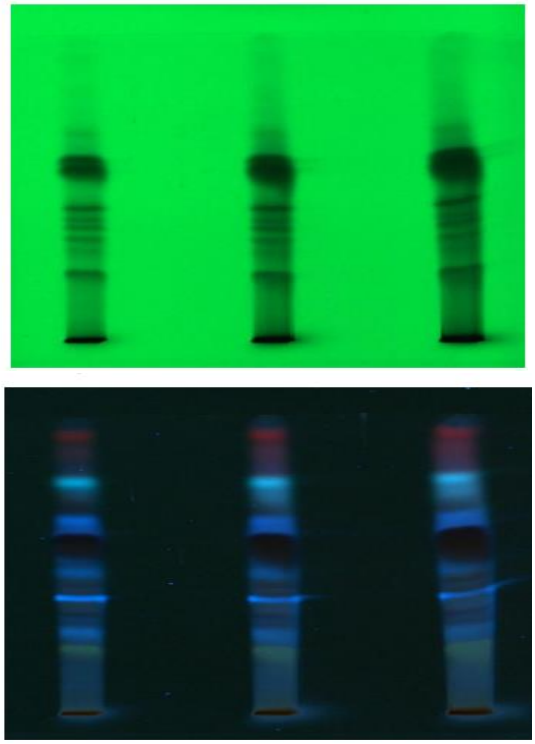

Fig 2. Chromatogram of F2

Table 2. HPTLC finger printing [at 254nm]

S. N.	F3		F4		F5	
	R_f Value	Area	R_f Value	Area	R_f Value	Area
1	0.22	10221.1	0.29	19719.3	0.15	1374.2
2	0.42	3411.8	0.40	1589.0	0.34	266.6
3	0.47	2289.9	0.48	8931.7	0.52	145.5
4	0.59	4211.1	0.53	6546.6	0.69	1124.7
5	0.71	3256.9	0.58	3285.0		
6	0.87	723.0	0.63	9657.1		

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7			0.76	8765.1		
8			0.90	4507.3		
9			0.97	190.1		

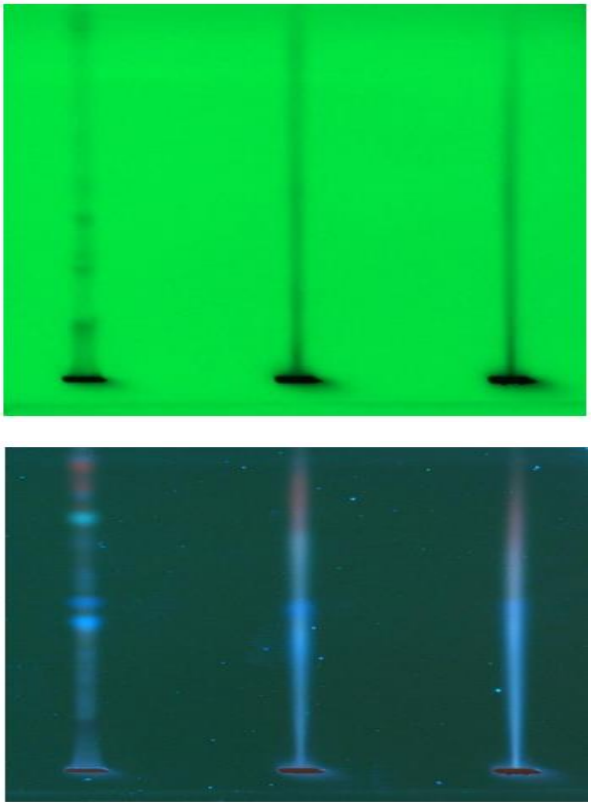
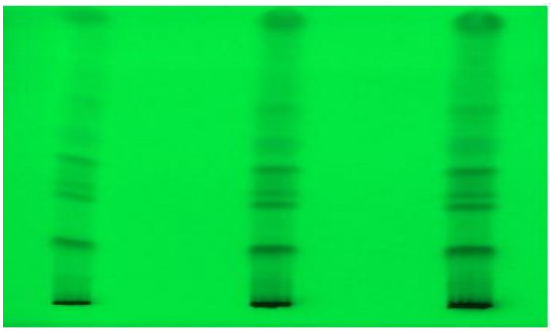


Fig 3. Chromatogram of F3



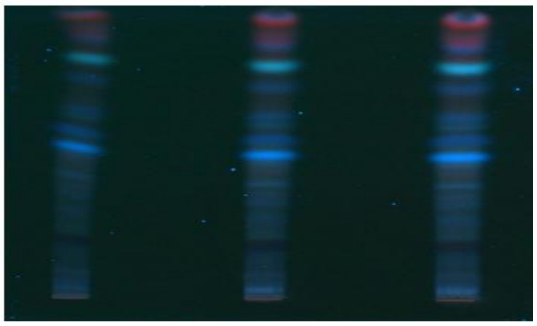


Fig4. Chromatogram of F4

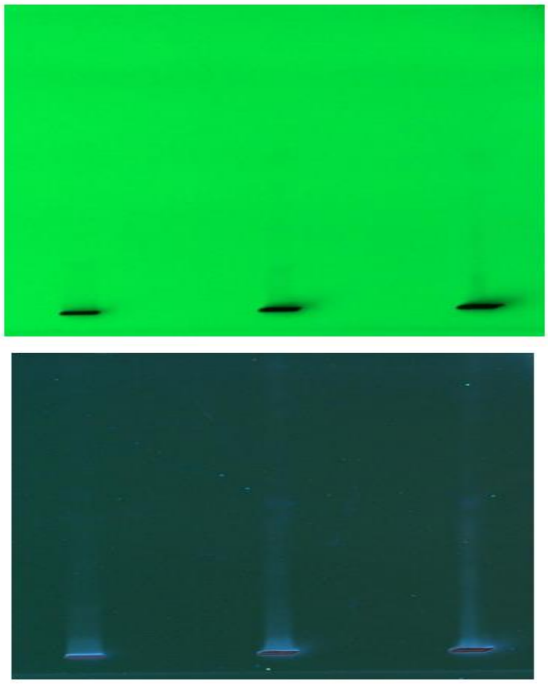


Fig 5. Chromatogram of F5

Discussion At 254 nm, many conjugated phytochemicals—especially alkaloids, phenolics, flavonoids—absorb strongly, making their spots appear as *dark bands* on plates coated with F_{254} silica gel.in the HPTLC analysis of sample there was many unknow compound found in the sample. F1 had 10 unknown compound, F2 & F4 had 9 compound, F3 had 6 and F5 had 4 compounds. HPTLC has already demonstrated utility in quantifying antioxidant markers (chlorogenic acid, rutin) and probing bioactive bands in mulberry and fig extracts. Future work includes establishing chromatographic libraries, integrating bioactivity screenings, optimizing extraction processes, and

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applying fingerprints for quality control in industrial uses.

CONFLICT OF INTEREST

None.

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