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COMPARATIVE HPTLC FINGERPRINTING OF AYURVEDIC MEDICINAL DRUGS: PUSHKARMULA, PALASHA

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ABSTRACT:
Background: Ayurvedic medicinal drugs need analytical methods to confirm their identity, check their quality, and ensure standardization. This is important because these drugs contain mixtures of plant-based chemicals. These chemicals can create patterns when analysed, which are called chromatographic profiles. Performance thin-layer chromatography, or HPTLC, is a powerful method for creating these fingerprint patterns. It helps in identifying the chemical signature of each drug. This technique is especially useful for medicines because of their complex composition. HPTLC provides an accurate way to compare different samples and verify their authenticity.
Objective: The present study aimed to comparatively evaluate the HPTLC fingerprint profiles of two Ayurvedic medicinal drugs, Pushkarmula and Palasha, with emphasis on their chromatographic characteristics and differentiation.
Methodology: Methanolic extracts of Pushkarmula and Palasha were analysed by HPTLC on silica gel 60 F_{254} plates. Chromatographic development was carried out using toluene: ethyl acetate: methanol: formic acid (7:3:3:0.2, v/v/v/v) as the mobile phase. Samples were applied at 5 and 10 µL, and chromatographic profiles were evaluated by densitometric scanning at 254 nm and 366 nm. The number of peaks, Rf values, and percentage peak areas were recorded for comparative fingerprint analysis.
Results: Distinct HPTLC fingerprint patterns were obtained for Pushkarmula and Palasha at both detection wavelengths. At 254 nm, Pushkarmula showed 6 peaks at 5 µL and 5 peaks at 10 µL, whereas Palasha showed 8 and 7 peaks, respectively. Characteristic differences in Rf values and peak-area distribution were observed between the two drugs. Pushkarmula exhibited a prominent peak at Rf 0.583 (55.76% area) at 5 µL, while Palasha showed prominent responses around Rf 0.633 (31.48%) at 5 µL.
Conclusion: The study demonstrates that HPTLC fingerprinting provides distinguishable chromatographic profiles for Pushkarmula and Palasha. The observed fingerprint characteristics may serve as useful analytical parameters for their identification, differentiation, and quality assessment.

KEYWORDS: HPTLC; Fingerprinting; Pushkarmula; Palasha; Ayurvedic medicinal drugs; Chromatographic profiling; Standardisation.

INTRODUCTION

Ayurvedic medicine relies extensively on medicinal plant materials as raw drugs and therapeutic ingredients.1 The identity and quality of these drugs are closely associated with the botanical source, plant part, geographical origin, processing conditions, and chemical composition2. Consequently, reliable methods for authentication and quality assessment are essential to ensure consistency and reproducibility of herbal medicinal materials3. Conventional pharmacognostic and physicochemical evaluation provides important information regarding the identity and quality of crude drugs; however, these approaches may not adequately characterise the complex mixture of chemical constituents present in medicinal plants4. Chromatographic fingerprinting therefore provides an important complementary approach for establishing characteristic chemical profiles.5

Pushkarmula, traditionally identified with Inula racemosa Hook. f., is an important Ayurvedic medicinal drug6. The need for reliable authentication of Pushkarmula has been highlighted by recent quality-control research, particularly because substitution or adulteration involving morphologically related medicinal roots can create challenges in maintaining raw-drug identity.7 Recent investigations have demonstrated that HPTLC and other chromatographic techniques can provide useful chemical fingerprints for authentication and quality control of I. racemosa.8

Palasha, generally identified as Butea monosperma (Lam.) Taub., is another traditionally important medicinal plant used in Ayurveda 9. Previous investigations have employed HPTLC fingerprinting for B. monosperma and reported characteristic chromatographic profiles that can contribute to crude-drug identification and standardisation10. HPTLC has also been applied to different plant parts and extracts of B. monosperma, demonstrating variability in the number and distribution of chromatographic bands depending on the material and analytical conditions11.

Research problem
Limited comparative chromatographic information may make the reliable differentiation and characterisation of Palasha and Pushkarmula challenging.
Therefore, comparative HPTLC fingerprinting is required to establish their characteristic chromatographic profiles under standardised conditions.

Significance of the study
The present study is significant, as it establishes comparative HPTLC fingerprint profiles of Pushkarmula and Palasha under standardized chromatographic conditions12. Methanolic samples were analyzed on silica gel 60 F_{254} plates using toluene: ethyl acetate: methanol: formic acid (7:3:3:0.2, v/v/v/v) as the mobile phase, followed by densitometric scanning at 254 nm and 366 nm 13. The resulting Rf values, peak distribution, and relative peak areas provide characteristic chromatographic profiles that may support the identification, differentiation, and quality assessment of these two Ayurvedic medicinal drugs 14.

Research objective
Primary Objective:
To develop and comparatively evaluate the HPTLC fingerprint profiles of Pushkarmula and Palasha under standardized chromatographic conditions at 254 nm and 366 nm.

Specific Objectives:
- To develop HPTLC fingerprint profiles of Pushkarmula and Palasha.
- To compare the number and distribution of chromatographic peaks and their Rf values.
- To compare the relative peak areas of the detected chromatographic components.
- To assess the potential utility of HPTLC fingerprinting for the differentiation and quality assessment of Pushkarmula and Palasha.

Research hypothesis
H_1 (alternative hypothesis): Pushkarmula and Palasha possess distinguishable HPTLC fingerprint profiles under identical chromatographic conditions, allowing their chromatographic differentiation.
H_0 (null hypothesis): Pushkarmula and Palasha do not exhibit significant distinguishable HPTLC fingerprint characteristics under the selected analytical conditions.

Materials and Methods / Methodology
2.1 Research Design
The present study was designed as a comparative, laboratory-based analytical study to evaluate and compare the HPTLC fingerprint profiles of two Ayurvedic medicinal drugs, Palasha and Pushkarmula. The study involved preparation of the respective plant materials followed by HPTLC analysis under standardized chromatographic conditions. The chromatographic profiles were evaluated at 254 nm and 366 nm to characterize and differentiate the two medicinal drugs.

2.2 Study Samples
The study comprised two Ayurvedic medicinal drug samples: Palasha and Pushkarmula
The respective plant materials were collected and authenticated prior to analysis. Following authentication, the materials were processed into powder and extracted according to the laboratory's established procedure. The prepared extracts were subsequently subjected to HPTLC analysis.
The HPTLC report included two application volumes for each drug, namely 5 µL and 10 µL, allowing comparative evaluation of the chromatographic profiles. The report specifically assigned Palasha to Tracks 1 and 2 and Pushkarmula to Tracks 5 and 6.

2.3 Collection and Authentication of Plant Material
Palasha and Pushkarmula were collected from the specified source and authenticated prior to their use in the study. The collected plant materials were identified on the basis of their relevant botanical characteristics and authenticated by the designated authority/expert.
Date of collection: 09th August 2026
Authenticated botanical names:
Pushkarmula: Binomial name: Inula racemosa root
Family: Asteraceae
Palasha: Binomial name: Butea monosperma.
Family: Fabaceae
Uuthenticator/institution: Managing Director, President, Biotrik Organization Pvt. Ltd.

2.4 Preparation and Extraction of Samples
The authenticated samples of Palasha and Pushkarmula were dried and powdered prior to extraction. The powdered samples were extracted using methanol, and the resulting extracts were filtered and prepared for HPTLC analysis. The prepared methanolic samples were applied to the HPTLC plates at 5 and 10 µL for chromatographic evaluation.

2.5 HPTLC Instrumentation and Chromatographic Conditions
HPTLC analysis was performed using a Linomat 5 sample applicator and TLC Scanner 4, with data acquisition and evaluation performed using vision CATS software version 3.2.23095.1.
Chromatographic separation was carried out on Merck HPTLC Silica gel 60 F_{254} plates (100 × 100 mm). The solvent front was developed up to 80 mm.
The mobile phase consisted of:
Toluene : Ethyl acetate : Methanol : Formic acid (7:3:3:0.2, v/v/v/v).
The developing chamber was saturated for 20 min using a saturation pad. After development, the plate was dried for 5 min at room temperature.

2.6 Sample Application
The prepared methanolic extracts of Palasha and Pushkarmula were applied to the HPTLC plate using a Linomat 5 applicator.
Two application volumes, 5 µL and 10 µL, were used for each drug. Sample application was performed at a dosage speed of 150 nL/s, with a pre-dosage volume of 0.20 µL. The application position was maintained at Y = 8.0 mm, with the first track positioned at X = 15.0 mm and a track-to-track distance of 13.4 mm.

2.7 Chromatographic Development
The applied samples were developed in a 10 × 10 cm chromatographic chamber using the selected mobile phase of toluene, ethyl acetate, methanol and formic acid in the ratio of 7:3:3:0.2 (v/v/v/v).
The chamber was saturated for 20 min before development. The chromatographic plate was developed to a distance of 80 mm, after which it was dried for 5 min at room temperature.

2.8 Detection and Densitometric Scanning
The developed HPTLC plate was evaluated at two detection wavelengths.

2.8.1 Detection at 254 nm
The chromatographic bands were scanned in absorbance mode at 254 nm using a deuterium lamp. The scanning speed was 100 mm/s, with a data resolution of 100 µm/step and slit dimensions of 6 × 0.45 mm.

2.8.2 Detection at 366 nm
The developed plate was additionally scanned in fluorescence mode at 366 nm using a mercury lamp with a K400 filter. The reported scanning speed, data resolution and slit dimensions were maintained as specified in the HPTLC method.

2.9 HPTLC Fingerprint Evaluation
The chromatographic fingerprints obtained for Palasha and Pushkarmula were evaluated using the densitograms generated by visionCATS software. The chromatographic profiles were assessed based on the number of detected peaks, Rf values, peak height, peak area, percentage peak area, peak distribution, and overall fingerprint pattern. The resulting chromatographic characteristics were compared between Palasha and Pushkarmula at 254 nm and 366 nm.

2.10 Variables and Measures
The independent variables were the type of Ayurvedic medicinal drug (Palasha and Pushkarmula), application volume (5 and 10 µL), and detection wavelength (254 and 366 nm). The dependent variables included the number of chromatographic peaks, Rf values, peak height, peak area, percentage peak area, and characteristic HPTLC fingerprint pattern. These parameters were used for the comparative evaluation of the chromatographic profiles of Palasha and Pushkarmula.

2.11 Statistical and Data Analysis
The HPTLC data were subjected to descriptive and comparative chromatographic analysis. The number of peaks, Rf values, peak heights, peak areas and percentage peak areas obtained from the densitograms were recorded and compared between Palasha and Pushkarmula.
Comparisons were performed with respect to:
- Palasha versus Pushkarmula
- 5 µL versus 10 µL application volume
- 254 nm versus 366 nm detection

The results were presented in tabular and graphical/fingerprint form. Since the available HPTLC report provides chromatographic peak data rather than independent replicate measurements, the analysis was primarily descriptive and no inferential statistical test was applied.

2.12 Ethical Considerations
The study involved the analytical evaluation of plant-derived medicinal drug samples and did not involve human participants, human biological samples or experimental animals. Therefore, ethical approval for human or animal experimentation was not applicable to the present study.

RESULTS

3.1 HPTLC Fingerprint Profile

HPTLC fingerprinting was performed for Palasha and Pushkarmula at application volumes of 5 and 10 µL. The chromatographic profiles were evaluated by densitometric scanning at 254 nm and 366 nm. The number of chromatographic peaks, Rf values, peak heights, peak areas, and percentage peak areas were recorded from the respective densitograms.

3.1.1 HPTLC Profile at 254 nm

At 254 nm, the 5 µL Palasha sample showed eight chromatographic peaks, with peak Rf values ranging from 0.164 to 1.000. The highest percentage peak area was observed at Rf 0.633 (31.48%), followed by peaks at Rf 0.369 (21.97%) and 0.458 (19.04%). The 10 µL Palasha sample showed seven peaks, with peak Rf values ranging from 0.161 to 0.997. The highest percentage peak area was observed at Rf 0.343 (41.55%), followed by Rf 0.419 (23.39%).

The 5 µL Pushkarmula sample showed six chromatographic peaks, with peak Rf values of 0.201, 0.583, 0.725, 0.790, 0.843, and 0.940. The highest percentage peak area was observed at Rf 0.583 (55.76%). The 10 µL Pushkarmula sample showed five peaks, with peak Rf values of 0.014, 0.042, 0.204, 0.578, and 0.908. The highest percentage peak area was observed at Rf 0.042 (58.69%), followed by Rf 0.204 (18.81%).

Table 1. Comparative HPTLC fingerprint profile of Palasha and Pushkarmula at 254 nm

Sample	Application volume	No. of peaks	Rf value of major peak	Maximum area (%)
Palasha	5 µL	8	0.633	31.48
Palasha	10 µL	7	0.343	41.55
Pushkarmula	5 µL	6	0.583	55.76
Pushkarmula	10 µL	5	0.042	58.69

3.1.2 HPTLC Profile at 366 nm

At 366 nm, the 5 µL Palasha sample showed nine chromatographic peaks, with peak Rf values ranging from 0.040 to 0.981. The highest percentage peak area was recorded at Rf 0.608 (32.42%). The 10 µL Palasha sample showed eight peaks, with peak Rf values ranging from 0.214 to 0.999. The highest percentage peak area was observed at Rf 0.899 (33.52%).

The 5 µL Pushkarmula sample showed four chromatographic peaks, with peak Rf values of 0.035, 0.200, 0.576, and 0.908. The highest percentage peak area was observed at Rf 0.035 (58.81%). The 10 µL Pushkarmula sample showed six peaks, with peak Rf values of 0.171, 0.206, 0.588, 0.728, 0.787, and 0.847. The highest percentage peak area was observed at Rf 0.588 (50.90%), followed by Rf 0.171 (21.62%).

Table 2. Comparative HPTLC fingerprint profile of Palasha and Pushkarmula at 366 nm

Sample	Application volume	No. of peaks	Rf value of major peak	Maximum area (%)
Palasha	5 µL	9	0.608	32.42
Palasha	10 µL	8	0.899	33.52
Pushkarmula	5 µL	4	0.035	58.81
Pushkarmula	10 µL	6	0.588	50.90

3.2 ComparativePTLC Fingerprint Characteristics

The overall HPTLC fingerprint characteristics of Palasha and Pushkarmula at the two detection wavelengths are summarized in Table 3.

Table 3. comparative HPTLC fingerprint profile

Drug	Application volume	Peaks at 254 nm	Peaks at 366 nm
Palasha	5 µL	8	9
Palasha	10 µL	7	8
Pushkarmula	5 µL	6	4
Pushkarmula	10 µL	5	6

The densitometric profiles obtained at 254 nm and 366 nm are presented in Figures 1–4.

3.3 Statistical/Data Analysis

The HPTLC results were evaluated using descriptive chromatographic analysis. The number of peaks, Rf values, peak heights, peak areas, and percentage peak areas obtained from the densitograms were recorded and compared according to the drug, application volume, and detection wavelength.

No inferential statistical analysis was performed because the available HPTLC report contains chromatographi data for the analyzed tracks but does not provide sufficient independent replicate measurements for calculation of statistical significance.

DISCUSSION

HPTLC fingerprinting generated comparative chromatographic profiles for Palasha and Pushkarmula at 254 and 366 nm. Differences were observed in peak number, Rf values, peak distribution, and percentage peak areas at both application

volumes. Palasha showed 8 and 7 peaks at 254 nm and 9 and 8 peaks at 366 nm, whereas Pushkarmula showed 6 and 5 peaks at 254 nm and 4 and 6 peaks at 366 nm at 5 and 10 µL, respectively.

The major peaks also occurred at different Rf positions, indicating differences in the chromatographic profiles of the two drugs. Such multi-component chromatographic fingerprinting is useful for the comparative characterization and quality assessment of complex herbal materials.12,13 However, as individual peaks were not identified using reference standards, the present findings represent comparative fingerprint characteristics rather than compound-specific identification.

Overall, the developed HPTLC profiles provide a useful chromatographic basis for the differentiation and characterization of Palasha and Pushkarmula. Further studies involving marker standards, method validation, and independent samples may strengthen their application in quality control.

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