World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 6.916
Year - 2026
Volume: 5; Issue: 11
Page: 337-343

# HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) FINGERPRINTING OF *WRIGHTIA TINCTORIA* ROXB LEAVES

**Aiswarya T. Mohandas*[1], P. Y. Ansary[2], Greeshma K. C.[3]**

[1]PG scholar, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, Kerala, India.
[2]Professor and HOD, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, Kerala, India.
[3]Assistant Professor, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, Kerala, India.

***Article Received: 20 September 2026*** | | ***Article Revised: 11 October 2026*** | | ***Article Accepted: 01 November 2026***

***Corresponding Author: Aiswarya T. Mohandas**
PG scholar, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, Kerala, India.
**DOI:** https://doi.org/10.5281/zenodo.23945022

**How to cite this Article:** Aiswarya T. Mohandas, P. Y. Ansary, Greeshma K. C. (2026) HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC) FINGERPRINTING OF *WRIGHTIA TINCTORIA* ROXB LEAVES. World Journal of Pharmaceutical Science and Research, 5(11), 337-343.

Copyright © 2026 Aiswarya T. Mohandas | World Journal of Pharmaceutical Science and Research.
This work is licensed under creative Commons Attribution-NonCommercial 4.0 International license (CC BY-NC 4.0).

## ABSTRACT

**Background:** *Wrightia tinctoria* Roxb., a member of the Apocynaceae family, is a valuable extra-pharmacopeial plant in Ayurvedic practice, recognized for its anti-inflammatory properties and historical use in treating psoriasis. **Objective:** The study aims to conduct a HPTLC fingerprint profile of its swarasa (leaf juice). **Materials and Methods:** shade-dried leaf powders were subjected for the HPTLC analysis, the methanolic extract of the leaf juice was developed using a Toluene: Acetone: Methanol (8.9:0.9:0.2) solvent system, followed by densitometric scanning at 520 nm after derivatization with an anisaldehyde–sulphuric acid reagent. **Results:** The HPTLC fingerprinting profile displayed 12 distinct peaks, indicating a complex phytoconstituent profile. A major peak was identified at Rf ~0.88, confirming the presence of the active triterpenoid lupeol, which aligned with the standard lupeol peak at Rf ~0.89. **Conclusion:** The developed HPTLC profile successfully identified lupeol and serves as a reliable reference tool for the future quality control and standardization of *Wrightia tinctoria* Roxb.

**KEYWORD**: *Wrightia tinctoria* Roxb., High-Performance Thin-Layer Chromatography (HPTLC), Lupeol.

## INTRODUCTION

High-Performance Thin-Layer Chromatography (HPTLC) is an analytical technique used for standardisation of raw drugs. It helps with qualitative and quantitative analysis. It helps to identify the number of peaks in an extract when compared with a standard marker compound. So, the same drug in different extracts may represent a different number of peaks. A greater number of peaks means more phytoconstituents, and peak size represents the quantity of that phytoconstituent.

*Wrightia tinctoria* Roxb. belongs to the family Apocynaceae[1] and is one of the valuable medicinal plants used in Ayurvedic practice. It is considered an extra- pharmacopeial drug, not directly mentioned in classical Ayurvedic texts. In Hortus Malabaricus,[2] it is identified as "*Neelam pala*". In this work, Hendrik van Rheede and later commentator Dr K. S. Manilal emphasised the effectiveness of oil extracted from the leaves in treating psoriasis. Because of its strong anti-inflammatory and antimicrobial properties, almost every part of the tree especially the leaves hold therapeutic value.

Phytochemical investigations conducted in previous studies reported the presence of several specific classes of bioactive compounds. Terpenoids, which include β-Caryophyllene, flavonoids such as Flavone 4-OH, 5-OH; other vital constituents comprise vitamins, specifically γ-Tocopherol and δ-Tocopherol, alongside esters such as Eicosatrienoic acid methyl ester and Phytol palmitate. Finally, sugars are also present within the plant's chemical profile, such as the glucosides found in Flavone 4-OH, 5-OH, 7-di-O-glucoside. These constituents are primarily responsible for its recognised activities, such as anti-inflammatory and immunomodulatory effects.[3,4]

In the present study, HPTLC fingerprinting of swarasa of leaves of *Wrightia tinctoria* Roxb is analysed.

## MATERIALS AND METHODS

### Collection of Drug

Fresh leaves of *Wrightia tinctoria* Roxb. were collected in the morning and cleaned with running water to remove impurities. The leaves are layed in a thin, single layer on clean cloths to ensure that the drug is free from impurities and contamination. The collected drug specimen's identification was done by the faculty of the Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, and plant authentication has been done at St. Albert's College (Autonomous), Ernakulam, Department of Botany, and the voucher number is 628Shade dry

### Preparation of Drug

### HPTLC of swarasa

*Swarasa* was prepared by crushing the freshly obtained leaves of *Wrightia tinctoria* Roxb. and extracting them immediately by squeezing through a cloth.

## MATERIALS AND METHODOLOGY

### Materials Required

*Wrightia tinctoria* Roxb. leaf swarasa, lupeol standard, methanol, toluene, acetone, anisaldehyde–sulphuric acid reagent, 5 × 10 cm silica gel 60 F254 precoated TLC plates (E. Merck, 0.2 mm thickness on aluminium sheet), CAMAG Linomat V Automatic Sample Spotter, 100 µL Hamilton syringe, CAMAG glass twin trough chamber (5 × 10 cm), CAMAG TLC scanner 3, WINCATS software, UV light source, and Tungsten lamp.

### Procedure

### HPTLC profile

For the HPTLC analysis, the test solution was prepared by macerating 0.5g of the sample in 10 ml of methanol for 6 hours. The extract was filtered, evaporated to dryness, and the resulting residue was redissolved in 15 ml of methanol. A solvent system of Toluene: Acetone:Methanol (8.9:0.9:0.2) was prepared and used to saturate a twin trough chamber for 30 minutes. After the application syringe was washed twice with methanol, 8 µl of the test extract and 4 µl of the lupeol standard were applied onto the HPTLC plate, which was then developed in the saturated chamber until the

solvent front reached a distance of 8 cm. Once developed, the plate was allowed to air-dry completely and was examined under UV light at 254 nm and 366 nm to record the Rf values and colours of the resolved bands. Finally, the plate was placed in the scanner, the software parameters were configured, and the tracks were scanned at 425 nm, followed by an absorption reflection mode scan at 520 nm using a Tungsten lamp. All tracks were scanned to capture their UV spectra, a fingerprint was generated for each track, and the resulting spots were compared in the spectrum display.

## RESULTS

### HPTLC finger printing profile

HPTLC plate observed at 254 nm, 366 nm and after derivatization (visible light). Distinct bands were observed at different wavelengths. After derivatization, colored bands appeared, indicating the presence of triterpenoids such as lupeol.

Densitometric scanning was performed at 520 nm after derivatization with anisaldehyde–sulphuric acid reagent using a CAMAG TLC scanner. The densitogram showed multiple peaks corresponding to different phytoconstituents present in the extract.

### Quantitative Data

#### a. Area and peaks of leaf juice of *Wrightia tinctoria* at 520 nm

HPTLC fingerprinting profile of leaf juice of *Wrightia tinctoria* showed 12 peaks at the wavelength of 520 nm in a total area of 36081.5 AU. These 12 peaks were defined at the maximum Rf value of -0.03 with area 8409.1 AU, maximum Rf value of 0.04 with area 164.9 AU, maximum Rf value of 0.11 with area 769.5 AU, maximum Rf value of 0.16 with area 614.7 AU, maximum Rf value of 0.21 with area 1217.2 AU, maximum Rf value of 0.35 with area 1985.5 AU, maximum Rf value of 0.49 with area 4961.1 AU, maximum Rf value of 0.57 with area 5119.5 AU, maximum Rf value of 0.69 with area 3076.9 AU, maximum Rf value of 0.81 with area 3604.4 AU, maximum Rf value of 0.88 with area 5651.7 AU, and maximum Rf value of 1.18 with area 506.9 AU respectively.

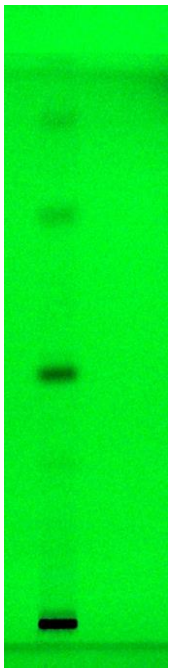
#### b. Area of peak of lupeol at 520 nm

The densitometric analysis of the standard lupeol at 520 nm revealed four distinct peaks. Among these, the third peak was found to be the major peak with a maximum Rf value of 0.89, exhibiting the highest peak height (615.1) and percentage area (94.42%), indicating the presence of lupeol as the major constituent. The other minor peaks were observed at Rf values of -0.03, 0.12, and 1.22 with percentage areas of 0.81%, 3.51%, and 1.26% respectively, which may correspond to impurities or minor components. The major peak (Peak 3) was therefore considered as the reference peak for lupeol in further analysis.

### Identification of Lupeol

Lupeol was identified in the sample at Rf ~0.88 by comparison with standard lupeol at Rf ~0.89 using HPTLC analysis at 520 nm.

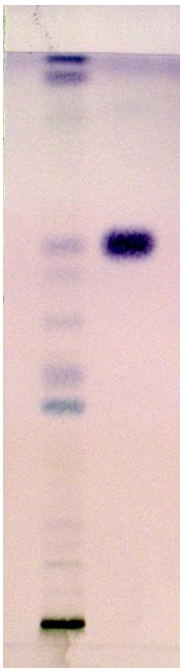
HPTLC fingerprinting profile of Swarasa of leaves of *Wrightia tinctoria* leaf is shown in below.



**Picture 1: HPTLC fingerprinting profile of Swarasa of leaves of *Wrightia tinctoria* leaf observed at 254 nm**



**Picture 2: HPTLC fingerprinting profile of Swarasa of leaves of *Wrightia tinctoria* leaf plate observed at 366 nm**



**Picture 3: HPTLC fingerprinting profile of Swarasa of leaves of *Wrightia tinctoria* leaf**

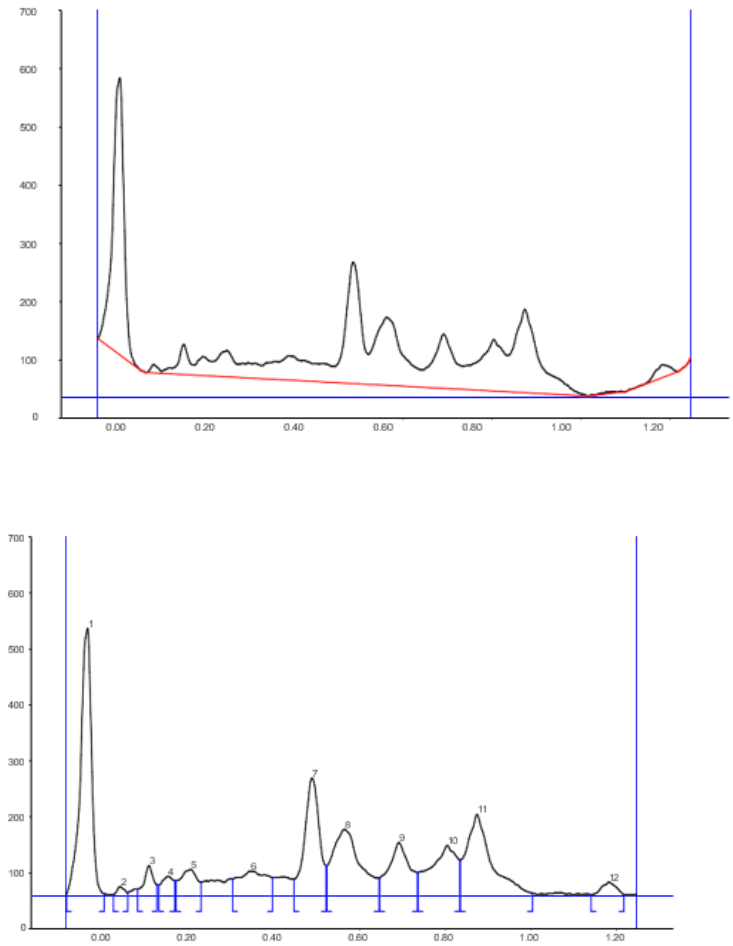



**Diagram 1(a &b): Densitogram swarasa of leaves of *Wrightia tinctoria* Roxb.**

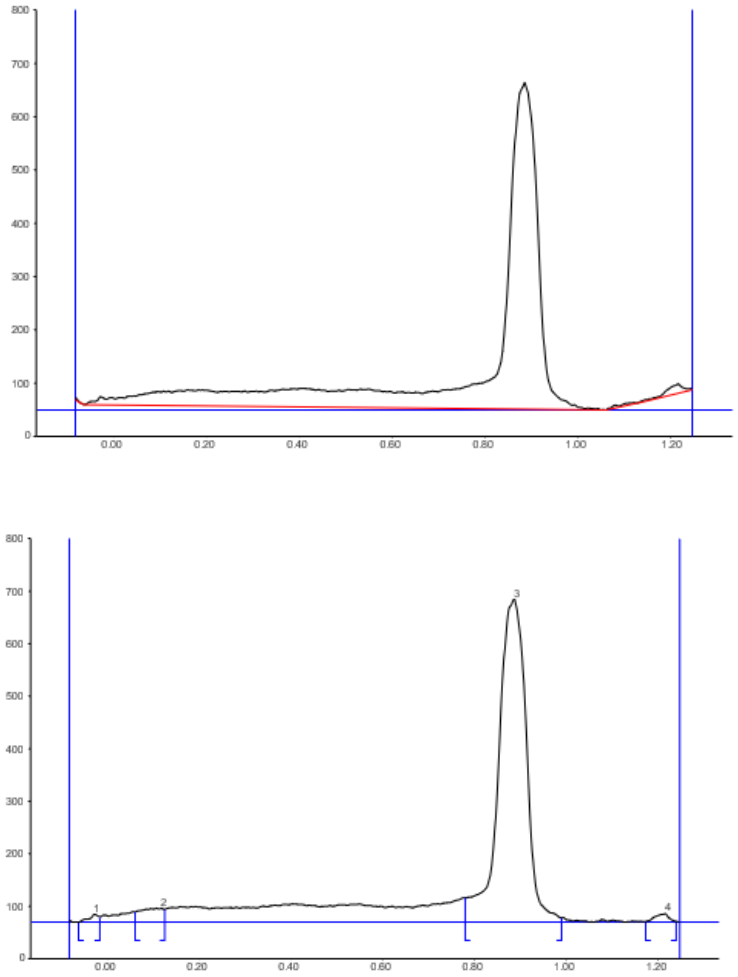



**Diagram 2 (c & d): Densitogram of Standard Lupeol**

Peak table of sample at 520 nm Peak table showing Rf, peak height, peak area and % area at 520 nm is shown in the table.

**Table 1: Peak table of sample at 520 nm Peak table showing Rf, peak height, peak area and % area at 520 nm.**

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.08 | 1.1 | -0.03 | 476.0 | 35.69 | 0.01 | 2.0 | 8409.1 | 23.31 | unknown * |
| 2 | 0.03 | 0.2 | 0.04 | 14.5 | 1.09 | 0.06 | 3.0 | 164.9 | 0.46 | unknown * |
| 3 | 0.09 | 9.8 | 0.11 | 52.1 | 3.91 | 0.13 | 17.8 | 769.5 | 2.13 | unknown * |
| 4 | 0.14 | 17.3 | 0.16 | 32.7 | 2.45 | 0.17 | 25.3 | 614.7 | 1.70 | unknown * |
| 5 | 0.17 | 25.1 | 0.21 | 45.2 | 3.39 | 0.23 | 22.2 | 1217.2 | 3.37 | unknown * |
| 6 | 0.31 | 28.3 | 0.35 | 42.0 | 3.15 | 0.40 | 30.6 | 1985.5 | 5.50 | unknown * |
| 7 | 0.45 | 27.7 | 0.49 | 208.2 | 15.61 | 0.52 | 51.6 | 4961.1 | 13.75 | unknown * |
| 8 | 0.53 | 51.8 | 0.57 | 116.5 | 8.73 | 0.65 | 29.7 | 5119.5 | 14.19 | unknown * |
| 9 | 0.65 | 30.2 | 0.69 | 93.0 | 6.97 | 0.74 | 39.9 | 3076.9 | 8.53 | unknown * |
| 10 | 0.74 | 39.9 | 0.81 | 87.9 | 6.59 | 0.84 | 62.1 | 3604.4 | 9.99 | unknown * |
| 11 | 0.84 | 62.1 | 0.88 | 143.2 | 10.73 | 1.01 | 2.6 | 5651.7 | 15.66 | unknown * |
| 12 | 1.14 | 0.4 | 1.18 | 22.6 | 1.69 | 1.22 | 0.3 | 506.9 | 1.40 | unknown * |

Peak table of standard lupeol at 520 nm is shown in the table.

**Table 2: Peak table of standard lupeol at 520 nm**.

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.06 | 0.2 | -0.03 | 15.0 | 2.24 | -0.02 | 10.5 | 222.1 | 0.81 | unknown * |
| 2 | 0.06 | 19.3 | 0.12 | 26.8 | 3.98 | 0.13 | 24.1 | 967.2 | 3.51 | unknown * |
| 3 | 0.78 | 46.3 | 0.89 | 615.1 | 91.37 | 0.99 | 8.3 | 26014.6 | 94.42 | unknown * |
| 4 | 1.17 | 1.0 | 1.22 | 16.2 | 2.41 | 1.24 | 2.1 | 347.2 | 1.26 | unknown * |

## DISCUSSION

### High profile thin layer chromatography (HPTLC) finger printing profile

HPTLC fingerprinting profile of *Wrightia tinctoria* Roxb. leaf juice (swarasa) was carried out in the present study. In a previous research work, methanolic extract of *Wrightia tinctoria* Roxb. leaves showed six spots in the chromatogram and the presence of lupeol was confirmed at an Rf value 0.77. HPTLC fingerprinting profile for this study of swarasa of leaves of *Wrightia tinctoria* Roxb. was done in silica gel plates and mobile phase consisting of toluene:acetone:methanol (8.9:0.9:0.2) and detection at 520 nm derivatization with anisaldehyde–sulphuric acid reagent. The separated components were identified by their Rf values and densitometric peaks. A total of 12 peaks were seen in the chromatogram thus presence of multiple phytoconstituents. Each peak corresponds to a chemical component. The difference in number of peaks of the previous study (six spots) may be because of the differences in extraction method and solvent system used. Here in this study, a prominent peak at Rf value 0.88, which is the lupeol standard peak observed at Rf value 0.89. This explains that lupeol is present in the swarasa of *Wrightia tinctoria*. The variation in Rf values in the earlier reported value (0.77) may be due to different mobile phase composition, detection wavelength, and derivatization reagent. The use of anisaldehyde–sulphuric acid reagent in the present study enhances the visualization of terpenoid compounds, thereby providing better specificity for identification of lupeol. Thus, the HPTLC fingerprint developed here can be considered as a reliable tool for identification, quality control, of *Wrightia tinctoria* Roxb. leaf drug.

## CONCLUSION

HPTLC analysis showed multiple peaks, which means the drug contains many chemical compounds. A major compound, lupeol, was identified, which is known for anti-inflammatory activity. This confirms that the drug has active components responsible for its effect. The HPTLC profile obtained in this study can be used as a reference for future quality control and standardization.

## ACKNOWLEDGEMENT

I am sincerely grateful to Dr. P.Y. Ansary, Professor and Head, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, for his invaluable guidance, encouragement, and insightful feedback, which greatly contributed to the successful completion of this study. I would like to express my gratitude to Dr Reshma P John and Dr Greeshma K C, Assistant Professors, Department of Dravyaguna Vigyana, Government Ayurveda College, Tripunithura, for their constant support. I would like to extend my thanks to all the staff of CARe KERALAM Ltd., Thrissur, for their assistance and cooperation, which played a crucial role in the smooth execution of this study.

### Conflicts of Interest

There are no Conflicts of Interest

**Financial support and Scholarship**

None

**REFERENCES**

1. Hooker JD. Flora of British India. Reprint ed, Vol. 7, Bishen Singh Mahendra Pal Singh, Dehradun, 1983, 653.
2. Van Rheede H, Manilal KS (Trans). Hortus Malabaricus. Reprint ed, Department of Publications University of Kerala, Thiruvananthapuram, 2008; Vol. 9: 6-9.
3. Soumya A, Sachdev K, Beena P. Conventional and LC-MS based phytochemical analysis of *Wrightia tinctoria* Roxb. leaf ethanolic extract. Int J Pharm Sci, 2026; 4(6): 288-299.
4. Rajkumar S, Sathyaprabha G, Mathanmohun M. Phytoconstituents profiling and antioxidant potential of *Wrightia tinctoria* Roxb. Curr Bot, 2021; 12: 68-74.