

**PHYTOCHEMICAL PROFILING, CHROMATOGRAPHIC
FINGERPRINTING AND IN VITRO ANTIBACTERIAL EVALUATION
OF METHANOLIC LEAF EXTRACT OF WRIGHTIA TINCTORIA
(ROXB.) R.BR.**

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ABSTRACT

Wrightia tinctoria (Roxb.) R.Br. is a medicinal plant of the family Apocynaceae with a history of traditional use in India. The present study evaluated the leaves of *W. tinctoria* collected from Vazhani, Thrissur, Kerala, using phytochemical, chromatographic, antibacterial and spectroscopic approaches. Shade-dried leaves were powdered and extracted by continuous Soxhlet extraction with methanol. The extract was subjected to qualitative phytochemical screening, thin-layer chromatography (TLC), silica-gel column chromatography and high-performance thin-layer chromatography (HPTLC), followed by disc-diffusion testing against *Staphylococcus aureus* and *Escherichia coli*. Fourier-transform infrared (FTIR) and ultraviolet-visible (UV-visible) spectroscopy were used for spectral profiling. Qualitative screening detected deoxy sugars, triterpenoids, carbohydrates, reducing sugars, glycosides,

lkaloids, flavonoids, amino acids and steroids, whereas proteins were not detected. Column chromatography yielded seven fractions. HPTLC comparison showed bands with R_f values

comparable to rutin, gallic acid, ursolic acid, isatin, β -sitosterol, quercetin and an indole-alkaloid/indigotine-associated reference. Disc-diffusion testing produced inhibition zones of 8–30 mm under the reported test conditions. FTIR showed characteristic bands at 3329, 2925, 2854, 1720, 1653, 1617 and 1045 cm^{-1} , while UV-visible analysis showed an absorption maximum at 664 nm. The findings provide a preliminary phytochemical and chromatographic profile of *W. tinctoria* leaves and support further standardized chemical characterization and antimicrobial investigation.

KEYWORDS: *Wrightia tinctoria*; phytochemical screening; TLC; HPTLC; antibacterial activity; FTIR; UV-visible spectroscopy.

GRAPHICAL ABSTRACT

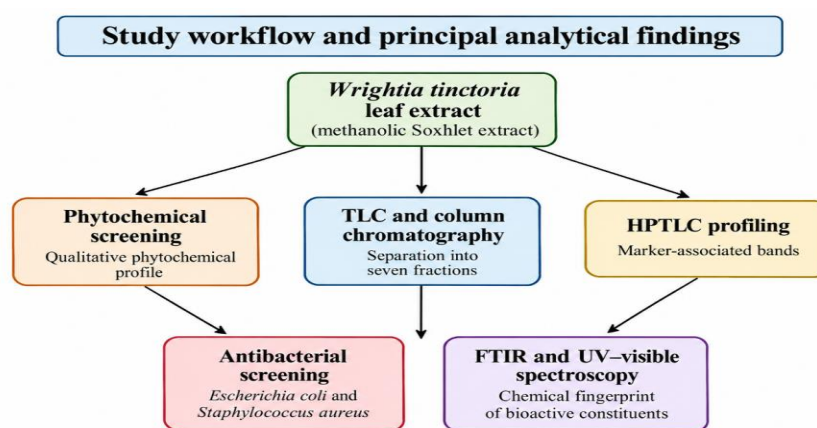


Figure 1: Study workflow and principal analytical findings.

1. INTRODUCTION

Wrightia tinctoria (Roxb.) R.Br. is a medicinal plant belonging to the family Apocynaceae and is traditionally used in India. Previous phytochemical investigations have reported phenolic, flavonoid, alkaloid, triterpenoid and steroid constituents from different parts of the plant, while antimicrobial studies have described activity against selected bacterial organisms.^[1-3,14,15,18,19] Recent investigations have further applied phytochemical profiling, chromatographic methods and mass-spectrometric approaches to characterize *W. tinctoria* extracts and associated bioactive constituents.^[14,15,19] Chromatographic fingerprinting is useful for documenting the chemical complexity of botanical extracts and for comparing samples with selected reference markers. HPTLC has been applied to *W. tinctoria* for fingerprint development and marker comparison.^[8] Accordingly, the present study was undertaken to characterize a methanolic leaf extract of *W. tinctoria* by qualitative

phytochemical screening, TLC, silica-gel column fractionation, HPTLC profiling, antibacterial disc diffusion and FTIR/UV-visible spectroscopy.

Chromatographic fingerprinting is useful for documenting the chemical complexity of botanical extracts and for comparing samples with selected reference markers. HPTLC is particularly useful for generating reproducible fingerprints that can support preliminary quality assessment and standardization. Accordingly, the present study was undertaken to characterize a methanolic leaf extract of *W. tinctoria* by qualitative phytochemical screening, TLC, silica-gel column fractionation, HPTLC profiling, antibacterial disc diffusion and FTIR/UV-visible spectroscopy.

2. MATERIALS AND METHODS

2.1 Plant material and authentication

Healthy and mature leaves of *W. tinctoria* were collected from a plantation at Vazhani, Thrissur district, Kerala, India, during March. The leaves were washed, shade-dried for approximately 10–14 days and powdered. The plant specimen was authenticated by Dr. Anto P.V., Assistant Professor, Research and P.G. Department of Botany, St. Thomas College, Thrissur, Kerala.

2.2 Extraction

Air-dried, coarsely powdered leaves were subjected to continuous Soxhlet extraction using methanol. The resulting extract was concentrated to dryness under reduced pressure. Soxhlet extraction is a conventional approach for recovering plant bioactive constituents and its efficiency depends on plant matrix, solvent and extraction conditions.^[9] The experimental results reported in this manuscript relate specifically to the methanolic extract.

2.3 Preliminary phytochemical screening

Qualitative chemical tests were performed for deoxy sugars, triterpenoids, carbohydrates, reducing sugars, glycosides, alkaloids, proteins, flavonoids, amino acids and steroids. The reactions were recorded qualitatively as positive or negative.

2.4 TLC and column chromatography

TLC was performed on silica-based plates using multiple solvent systems. The reported solvent systems were n-hexane: ethyl acetate (8: 2), methanol, benzene: chloroform: ethanol (4: 2: 1), methanol: water (5: 5) and methanol: ethyl acetate (5: 5), with appropriate

visualization reagents. The R_f value was calculated as the distance travelled by the solute divided by the distance travelled by the solvent front. The use of TLC/HPTLC fingerprinting is consistent with previously reported chromatographic work on *W. tinctoria*.^[8]

Silica-gel column chromatography was performed using n-hexane: methanol (8: 2) for the first column and methanol: ethyl acetate (5: 5) for the second column. Five fractions were obtained from the first column and two from the second, giving seven fractions in total. Column chromatography is a commonly used approach for fractionation and purification of plant secondary metabolites.^[10]

2.5 HPTLC analysis

HPTLC profiling was performed using pre-coated silica plates. Benzene: chloroform: ethanol (4: 2: 1) was used as the mobile phase, followed by UV visualization and densitometric recording of peak height, peak area and R_f values. Fractions were compared with reference standards to obtain tentative marker-associated assignments.

2.6 Antibacterial disc-diffusion assay

Antibacterial screening was performed against *Staphylococcus aureus* and *Escherichia coli* using Mueller–Hinton agar. Sterile 6-mm discs loaded with extract or reference drugs were placed on inoculated agar and incubated at 37 °C for 24 h. The diameter of the inhibition zone was recorded. The activity index was calculated as the ratio of the test inhibition zone to the corresponding standard inhibition zone. The disc-diffusion approach follows the general Kirby–Bauer principle.^[12]

2.7 FTIR and UV-visible spectroscopy

FTIR spectra were recorded over 4000–400 cm⁻¹. The reported characteristic bands were 3329, 2925, 2854, 1720, 1653, 1617 and 1045 cm⁻¹. UV-visible spectroscopy was performed over 400–800 nm, and the reported absorption maximum was 664 nm with an absorbance of 0.925. FTIR was used as a functional-group profiling technique rather than as a definitive method for individual compound identification.^[13]

2.8 Data Presentation

The experimental record did not report replicate number, standard deviation/standard error, confidence intervals or inferential statistical testing for the disc-diffusion measurements.

Accordingly, antibacterial findings are presented descriptively, and no unreported statistical significance is assigned.

3. RESULTS

3.1 Qualitative phytochemical profile

The methanolic leaf extract gave positive reactions for deoxy sugars, triterpenoids, carbohydrates, reducing sugars, glycosides, alkaloids, flavonoids, amino acids and steroids, whereas proteins were not detected (Table 1).

Table 1: Qualitative phytochemical screening of the methanolic leaf extract of *W. tinctoria*.

Phytoconstituent	Result
Deoxy sugars	+
Triterpenoids	+
Carbohydrates	+
Reducing sugars	+
Glycosides	+
Alkaloids	+
Proteins	—
Flavonoids	+
Amino acids	+
Steroids	+

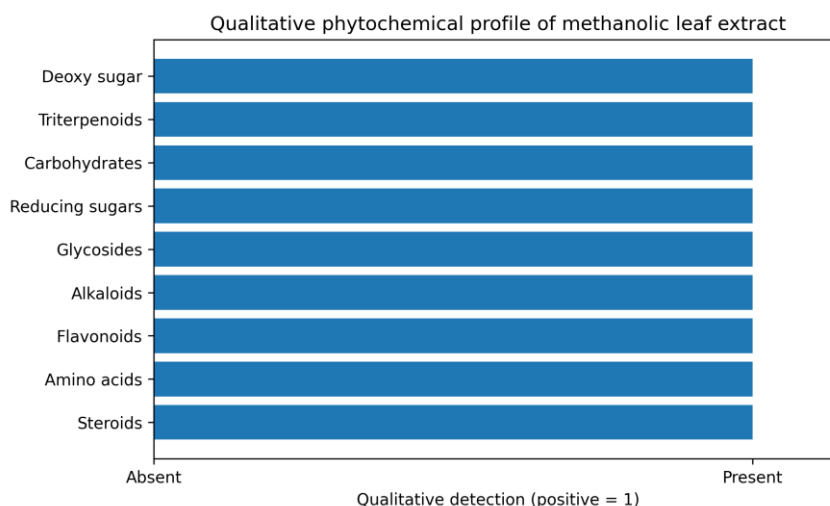


Figure 2: Qualitative phytochemical profile of the methanolic leaf extract.

3.2 TLC profiling and column fractionation

TLC produced distinct spots across the reported solvent systems. The highest number of spots was observed with benzene: chloroform: ethanol (4: 2: 1) and Dragendorff's reagent, for which three R_f values (0.54, 0.18 and 0.94) were reported. Column chromatography yielded

seven fractions using two solvent systems, providing material for subsequent HPTLC comparison.

Table 2: TLC profile of the methanolic leaf extract.

Solvent system	Visualization reagent	Spots	Reported Rf values
n-Hexane: ethyl acetate (8: 2)	Vanillin–sulfuric acid	1	0.54
Methanol	Ferric chloride	1	0.44
Benzene: chloroform: ethanol (4: 2: 1)	Dragendorff's reagent	3	0.54, 0.18, 0.94
Methanol: water (5: 5)	Ninhydrin	1	0.75
Methanol: ethyl acetate (5: 5)	Mayer's reagent	2	0.36, 0.90

Table 3: Silica-gel column chromatographic fractionation.

Column	Solvent system	Ratio	Volume	Fractions
1	n-Hexane: methanol	8: 2	100 mL	5
2	Methanol: ethyl acetate	5: 5	100 mL	2

3.3 HPTLC marker-associated profiling

HPTLC analysis provided a chemical fingerprint and tentative marker associations. Fraction 1 showed a rutin-associated band at Rf 0.44; fraction 2 showed gallic-acid-associated bands including Rf 0.36; fraction 3 showed ursolic-acid-associated bands at Rf 0.45; fraction 4 showed isatin-associated bands; fraction 5 showed β -sitosterol-associated bands at Rf 0.66; fraction 6 showed quercetin-associated bands around Rf 0.53; and fraction 7 showed indole-alkaloid-associated bands at Rf 0.54–0.59. The latter was interpreted in the original experimental record as being associated with indigotine. These assignments are considered tentative because definitive compound identification would require orthogonal characterization such as LC-MS/MS, NMR or isolated-standard confirmation.

Table 4: HPTLC marker-associated assignments for the column fractions.

Fraction	Reference marker	Reported/compared Rf	Interpretation
F1	Rutin	0.44	Rutin-associated
F2	Gallic acid	0.36 (standard 0.35)	Gallic-acid-associated
F3	Ursolic acid	0.45	Ursolic-acid-associated
F4	Isatin	≈0.42	Isatin-associated
F5	β -Sitosterol	0.66	β -Sitosterol-associated
F6	Quercetin	0.53 (standard 0.49)	Quercetin-associated
F7	Indole alkaloid	0.54–0.59	Indole-alkaloid/indigotine-associated

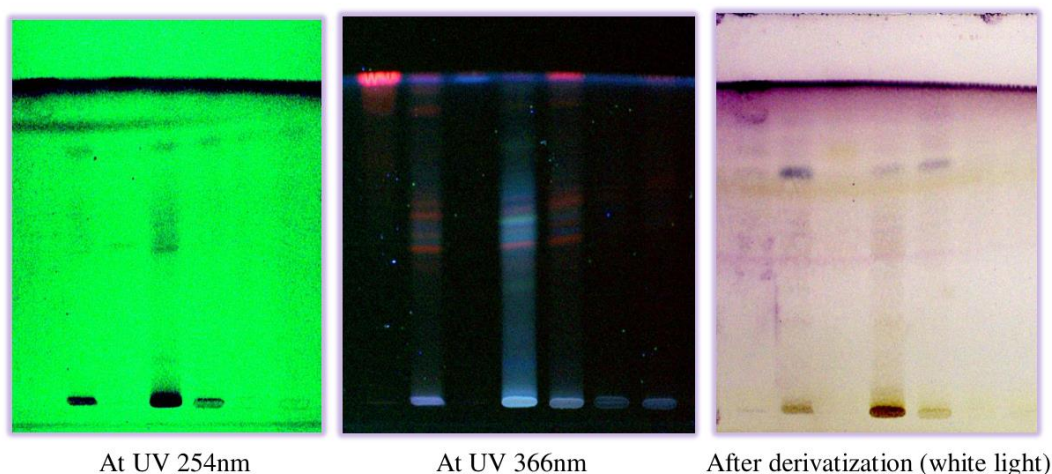


Figure 3: HPTLC fingerprint of the reported column fractions under UV 254 nm, UV 366 nm and after derivatization.

3.4 Antibacterial disc-diffusion activity

The methanolic extract produced measurable inhibition zones under the reported test conditions. The largest reported test zone was 30 mm against *S. aureus* when gentamicin was used as the corresponding reference. For *E. coli*, reported test zones were 8 mm with amoxicillin, 25 mm with gentamicin and 28 mm with diclofenac. The corresponding activity indices were 0.80, 1.30, 1.09 and 1.12, respectively (Table 5).

Table 5: Disc-diffusion inhibition zones and calculated activity indices.

Organism / reference	Test zone (mm)	Standard zone (mm)	Activity index
<i>E. coli</i> / Amoxicillin	8	10	0.80
<i>S. aureus</i> / Gentamicin	30	23	1.30
<i>E. coli</i> / Gentamicin	25	23	1.09
<i>E. coli</i> / Diclofenac	28	25	1.12

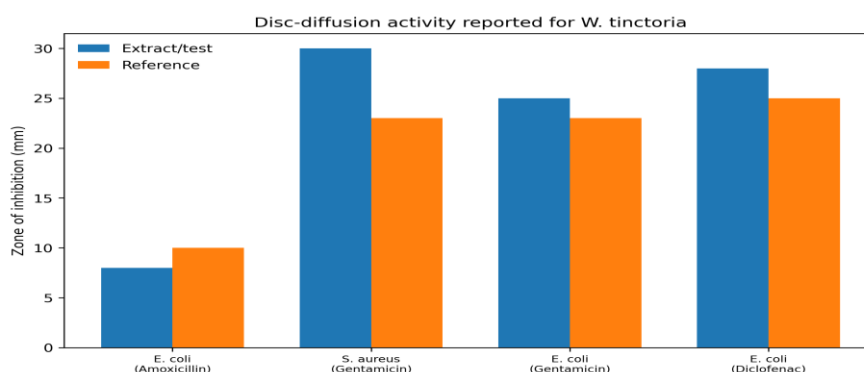


Figure 4: Reported disc-diffusion inhibition zones for the methanolic extract and corresponding reference conditions.

3.5 FTIR and UV-visible spectral observations

FTIR analysis showed a broad/strong band at 3329 cm^{-1} , reported as superimposed O–H/NH stretching, with additional bands at 2925 and 2854 cm^{-1} associated with C–H stretching. A band at 1720 cm^{-1} was assigned to C=O stretching, while bands at 1653 and 1617 cm^{-1} were associated with C=C/aromatic features. A band at 1045 cm^{-1} was assigned in the experimental record to C=N stretching. FTIR provides functional-group information but does not, by itself, establish individual compound identity.

Table 6: Reported FTIR bands and functional-group assignments.

Wavenumber (cm^{-1})	Assignment
3329	Superimposed O–H/NH stretching
2925	Asymmetric C–H stretching
2854	Symmetric C–H stretching
1720	C=O stretching
1653	C=C stretching / aromatic feature
1617	C=C stretching / aromatic feature
1045	C=N stretching

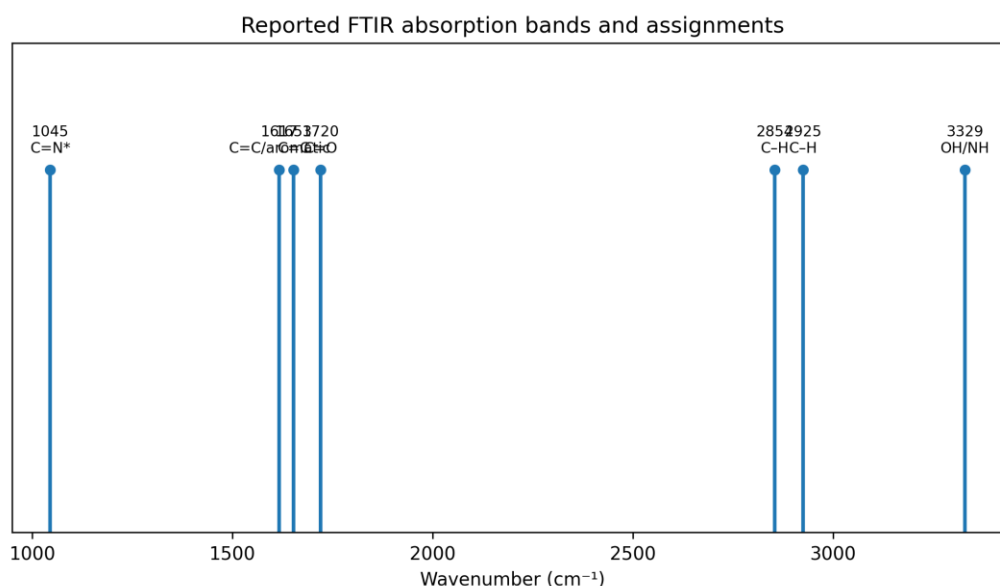


Figure 5: FTIR peak positions and assignments reported for the methanolic leaf extract.

UV-visible analysis of the extract over $400\text{--}800\text{ nm}$ showed a reported absorption maximum at 664 nm , with an absorbance of 0.925 .

4. DISCUSSION

The combined qualitative, chromatographic and spectroscopic findings indicate that the methanolic leaf extract contains multiple classes of phytoconstituents. The detection of

flavonoids, triterpenoids, steroids and alkaloids is consistent with the broader phytochemical literature on *W. tinctoria*.^[1,2] The HPTLC profile provides a practical chemical fingerprint and permits comparison with selected reference markers, which may be useful for preliminary quality-control studies.

The antibacterial screening demonstrated measurable inhibition under the reported conditions, with inhibition zones ranging from 8 to 30 mm. However, the reported experiments used different reference agents across test conditions and did not provide replicate measurements or measures of variability. In particular, diclofenac is not a conventional antibacterial reference agent; therefore, the corresponding activity-index value should not be interpreted as evidence of comparative antibacterial potency. The activity-index values should be interpreted as exploratory observations rather than definitive comparative antimicrobial efficacy. Earlier studies have also reported antimicrobial activity for *W. tinctoria*, while recent work has further examined its leaf extracts and antibacterial potential.^[5,6,17,19] Future studies should use standardized inoculum density, defined extract concentrations, biological replicates and MIC/MBC determinations.

The HPTLC marker associations should likewise be interpreted cautiously. Similar *R_f* values provide preliminary evidence of association but do not constitute definitive chemical identification. Future work should include quantitative marker estimation, LC-MS/MS and NMR characterization, and bioactivity-guided fractionation to establish compound identity and relate individual constituents to antibacterial activity.

5. STUDY LIMITATIONS

The available experimental record did not provide replicate numbers, measures of dispersion, inferential statistical analysis, extract yield, detailed inoculum standardization, extract concentration per disc, or MIC/MBC values. The use of different reference agents between test conditions, including diclofenac, further limits direct comparison of the reported inhibition zones. Accordingly, the antibacterial findings are presented descriptively and no unreported statistical significance is assigned.

6. CONCLUSION

The methanolic leaf extract of *Wrightia tinctoria* demonstrated a broad qualitative phytochemical profile and yielded seven chromatographic fractions with tentative HPTLC marker-associated bands corresponding to reference compounds including rutin, gallic acid,

ursolic acid, isatin, β -sitosterol, quercetin and an indole-alkaloid/indigotine-associated reference. Disc-diffusion testing demonstrated measurable inhibition against the reported bacterial test organisms, with inhibition zones of 8–30 mm under the reported conditions. FTIR and UV-visible observations provided complementary spectral information on the extract. Overall, the findings establish a preliminary phytochemical, chromatographic and antibacterial profile and support further standardized chemical characterization and antimicrobial evaluation.

7. ACKNOWLEDGEMENTS

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8. FUNDING

No external funding was reported for this study.

9. CONFLICT OF INTEREST

The authors declare no conflict of interest based on the information available for this study.

10. DATA AVAILABILITY

The data presented are derived from the experimental observations and records available for this study.

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