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Phytochemical profiling of bark of *Dalbergia sissoo* L. Roxb. Using HPTLC technique

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ABSTRACT

Dalbergia sissoo Roxb. commonly referred to as Indian Rosewood, is a well-recognized medicinal species belonging to the family Fabaceae. Traditionally, it has been employed in the management of various ailments such as inflammation, pain, diabetes, and microbial infections. The present investigation seeks to establish a chromatographic fingerprint of D. sissoo using High-Performance Thin Layer Chromatography (HPTLC) for qualitative phytochemical profiling and standardization purposes. HPTLC analysis of the plant extract revealed ten distinct, well-resolved peaks with Rf values ranging from 0.01 to 0.82, indicating the presence of a complex array of phytoconstituents. Among these, peak 10 accounted for the maximum area percentage (58.22%), signifying its predominance within the extract. The remaining peaks showed area percentages between 0.40% and 11.95%, corresponding to minor components. Although the individual compounds remain unidentified, the distinctive chromatographic pattern confirms the presence of multiple bioactive constituents in the extract.

Introduction

Phytochemicals are naturally occurring compounds in medicinal plants that contribute to their therapeutic efficacy against various diseases. Phytochemical screening serves as an essential approach to identify bioactive constituents present in different plant parts. In this context, an analysis of *Dalbergia sissoo* L. Roxb. was undertaken to explore its potential therapeutic applications as an antidiarrheal agent, a use long supported by traditional healers (Kalaskar et al., 2010). Since ancient times, medicinal plants have formed a foundational component of traditional healthcare systems and continue to serve as a valuable source for developing modern pharmaceutical agents (Gupta et al., 1994; Shukla, 2000). The pharmacological potential of these plants arises chiefly from their diverse secondary metabolites, including alkaloids, flavonoids, phenolics, terpenoids, and glycosides. Ensuring the safety, efficacy, and reproducibility of herbal

formulations requires rigorous standardization and quality control procedures. Among the various analytical tools available, High-Performance Thin-Layer Chromatography (HPTLC) has been recognized as a rapid, economical, and reliable technique for phytochemical profiling and fingerprinting of herbal extracts.

Dalbergia sissoo L. Roxb., a member of the Fabaceae family, is extensively distributed across the Indian subcontinent. The genus *Dalbergia* comprises approximately 300 species, of which 25 occur in India, with rosewoods being among the most valued members (Bharath et al., 2013). Traditionally, *D. sissoo* has been used in treating a range of ailments due to its anti-inflammatory, analgesic, antimicrobial, antidiabetic, and antioxidant properties. Ethnomedical reports indicate its use in conditions such as syphilis, gastrointestinal disorders, dysentery, skin infections, and fever. Its extracts also demonstrate inhibitory, larvicidal, and growth-regulating activities. Various plant parts including the bark, leaves, and heartwood are known to contain bioactive molecules like flavonoids, isoflavones, and phenolic compounds that contribute to these therapeutic effects (Vasudeva et al., 2009). Recent studies have extended its evaluation to biotechnological applications, including mesophilic anaerobic co-digestion of cattle manure with Malus domestica and D. sissoo for biomethane production (Awais et al., 2018). However, phytochemical composition may vary depending on geographical origin, environmental factors, and extraction procedures, emphasizing the necessity of chromatographic profiling for standardization (Niranjan et al., 2010).

HPTLC analysis provides a distinctive fingerprint profile that aids in the authentication of plant materials, detection of adulterants, and assessment of consistency across sample batches. In the present study, the bark extract of *D. sissoo* L. Roxb. was analyzed to determine its phytochemical constituents. The resulting chromatogram displayed ten distinct peaks with varying Rf values and area percentages, representing multiple phytochemical components. Among these, the major peak, accounting for 58.22% of the area, indicated the dominance of a principal compound, while the remaining peaks corresponded to minor constituents contributing to the plant's overall pharmacological activity. The findings highlight the significance of HPTLC as an effective method for the phytochemical characterization, quality evaluation, and potential isolation of bioactive compounds from Dalbergia sissoo L. Roxb.

Material and Methods:

Collection and Authentication of Plant Material

Fresh bark samples of *Dalbergia sissoo* L. Roxb. were collected from the local region and authenticated with reference to the Flora of Kolhapur District. The collected plant material was thoroughly washed with distilled water to eliminate dust and surface impurities, shade-dried at ambient temperature, and subsequently powdered using a mechanical grinder. The powdered bark was preserved in an airtight container until further analysis. A precisely weighed quantity of the powder (10 g) was extracted with methanol using a Soxhlet apparatus (alternatively by

maceration) for 6–8 hours. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The dried extract was stored at 4 °C until subsequent phytochemical evaluation (Asif et al., 2011; Shankar et al., 2012).

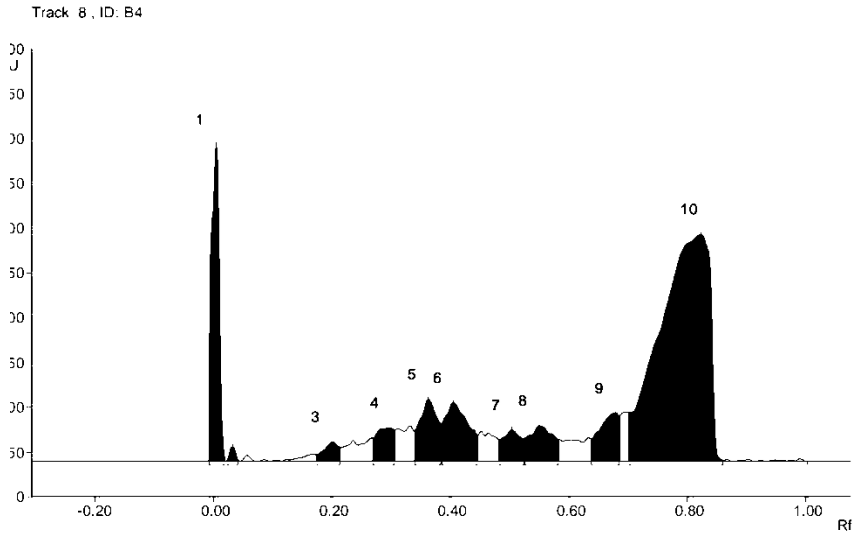
For HPTLC analysis, 10 mg of the dried extract was dissolved in 1 mL of methanol to yield a 10 mg/mL stock solution. The solution was sonicated for 5 minutes to ensure complete dissolution and filtered through a 0.45 µm membrane filter to remove particulates prior to application on HPTLC plates.

Chromatographic separation was carried out on pre-coated silica gel 60 F_{254} aluminum plates (Merck). Sample application was performed using a CAMAG Linomat applicator, and plate development was conducted in a CAMAG twin-trough chamber. The TLC Scanner III with winCATS software (CAMAG) was utilized for detection and analysis. Prior to application, the plates were pre-washed with methanol and activated at 110 °C for 5 minutes. Samples were applied as 6–8 mm bands at a constant rate under a nitrogen gas stream. Plate development was carried out over a distance of 80 mm using methanol as the mobile phase in a chamber pre-saturated for 20 minutes. The process was conducted at a temperature of 25 ± 2 °C.

Following development, the plates were air-dried and scanned at wavelengths of 254 nm and 366 nm. Densitometric measurements were recorded in absorbance mode to determine Rf values, peak heights, peak areas, and area percentages. Visualization of developed chromatograms was performed under both UV wavelengths (254 nm and 366 nm) using a TLC visualizer. The resulting densitograms were analyzed to construct an HPTLC fingerprint profile of the Dalbergia sissoo extract. Peaks were identified on the basis of their Rf values and relative area percentages. The number of peaks, corresponding Rf values, and their relative intensities provided qualitative insights into the composition and standardization of the bark extract, with major and minor constituents determined according to area distribution.

Morphology of Plant: Evergreen tree leaves 3-5Foliate, leaflets ovate or suborbicular, alternate, Flower subsessile, in axillary panicle shorter than the leaves corolla creamy white pods strap shaped often only 1 seeded.

Fig 1 HPTLC. Chromatogram of *Dalbergia sissoo* L Roxb. bark extract.



Fresh plant material of *Dalbergia sissoo* L Roxb.was collected from a local area and authenticated by a qualified botanist. The collected material was washed thoroughly with distilled water to remove dust and impurities, shade dried at room temperature, and then powdered using a mechanical grinder. The powdered sample was stored in an airtight container for further analysis.

Table : 1RF value of Leaf extract of *Dalbergia sissoo* L Roxb bark at 254 nm.

Sr.No	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area%
1	-0.01 Rf	0.00AU	0.00 Rf	352.9 U	37.48%Rf	0.02Rf	1.0	3227.6	11.95
2	0.02 Rf	0.00 AU	0.03 Rf	1 5.9 U	1.68% Rf	0.04 Rf	0.5	108.8	0.40
3	0.17 Rf	6.7 AU	0.20 Rf	21.2 U	2.25% Rf	0.21 Rf	15.1	398.8	1.45
4	0.27 Rf	25.5A U	0.30 Rf	36.7 U	3.90% Rf	0.31 Rf	34.3	843.6	3.07
5	0.34 Rf	33.3 AU	0.36 Rf	68.9 U	7.32% Rf	0.38 Rf	41.2	1548.5	5.64
6	0.39 Rf	41.7A U	0.41 Rf	64.9 U	6.90% Rf	0.45 Rf	28.5	1942.8	7.08
7	0.48 Rf	24.0 AU	0.50 Rf	34.8 U	3.70% Rf	0.52 Rf	25.2	803.9	2.93
8	0.53 Rf	25.4 AU	0.55 Rf	39.3 U	4.18% Rf	0.58 Rf	24.1	1195.2	4.36
9	0.64 Rf	24.7A U	0.68 Rf	53.7 U	5.70% Rf	0.69 Rf	50.6	1345.4	4.90
10	0.70 Rf	54.0 AU	0.82 Rf	253.2 U	26.89 Rf	0.86 Rf	0.8	15972.9	58.22

A precisely weighed portion of *Dalbergia sissoo* L. Roxb. bark powder (10 g) was extracted with methanol using a Soxhlet apparatus (alternatively by maceration) for 6–8 hours. The resulting extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator. The concentrated extract was subsequently dried and stored at 4 °C until further use for HPTLC analysis. A known quantity of the dried extract (10 mg) was dissolved in 1 mL of methanol to prepare a solution with a concentration of 10 mg/mL. The

solution was sonicated for 5 minutes to achieve complete dissolution and filtered through a 0.45 µm membrane filter to remove particulate impurities before chromatographic application.

HPTLC profiling was performed using a CAMAG HPTLC system integrated with winCATS software. The pre-coated silica gel 60 F_{254} plates were pre-washed with methanol and activated at 110 °C for 5 minutes before use. Samples were applied as 6–8 mm bands at a uniform rate using nitrogen gas. Plate development was carried out in a twin-trough chamber pre-saturated with the mobile phase for 20 minutes. After development, plates were air-dried and scanned at wavelengths of 254 nm and 366 nm using a TLC scanner. Densitometric scanning was performed in absorbance mode to determine Rf values, peak heights, peak areas, and corresponding area percentages.

The chromatograms were visualized under UV light at 254 nm and 366 nm using a TLC visualizer. The resulting densitograms were employed to generate the HPTLC fingerprint profile of the extract. Individual peaks were identified based on their Rf values and area percentages, providing qualitative insight into the phytochemical composition. All experiments were conducted in triplicate, and the results are presented as mean ± standard deviation to ensure methodological accuracy and reproducibility.

Result and Discussion:

The HPTLC profile exhibited ten distinct and well-resolved peaks, each corresponding to different phytoconstituents of varying polarity. The densitogram revealed a complex chromatographic pattern, suggesting the presence of multiple bioactive compounds within the methanolic extract. Peaks 1 and 10 displayed the highest heights and area percentages, indicating these as the predominant constituents. The major peak, characterized by a substantial area percentage, is likely derived from a non-polar or weakly polar compound possibly belonging to classes such as terpenoids, steroids, fatty acid derivatives, or other lipophilic molecules. The relative peak heights and areas represent the proportional abundance of both major and minor phytochemical constituents present in the extract.

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation to ensure reproducibility and reliability of the method.

Table 2 Interpretation of Individual Peaks

Sr No	Peak	Rf Range	Interpretation
1	1–3	–0.01–0.20	Highly polar compounds (e.g., sugars, phenolics)
2	4–8	0.27–0.55	Moderate polar phytochemicals (flavonoids, glycosides)
3	9–10	0.64–0.86	Less polar/lipophilic compounds (terpenoids, steroids)

It indicates the presence of a major non-polar or weakly polar compound, possibly terpenoids, steroids, fatty acid derivatives, or other lipophilic constituents.The height and area of peaks

reflect the relative concentration of compounds. Peak 1 and Peak 10 dominate the chromatogram, suggesting they are the major constituents of the sample.

Conclusion

The developed HPTLC method demonstrated clear separation, reproducibility, and reliability, establishing its suitability for the qualitative phytochemical profiling of *Dalbergia sissoo* L. Roxb. Although individual compounds were not structurally identified in the present study, the characteristic fingerprint obtained serves as a reference standard for the authentication, quality assessment, and standardization of *D. sissoo*-based herbal formulations. The study reinforces the medicinal significance of *Dalbergia sissoo* Roxb. and highlights the value of HPTLC as a rapid, economical, and dependable analytical approach for preliminary phytochemical evaluation. The established fingerprint profile provides a solid foundation for future investigations focusing on the isolation, identification, and pharmacological analysis of its bioactive constituents. The number of peaks, Rf values, peak heights, and area percentages were recorded and interpreted. The major and minor phytoconstituents were identified based on area percentage, indicating their relative abundance in the extract.

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