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Qualitative alkaloid profiling and solvent optimization in *Catharanthus roseus* (Alba) using thin layer chromatography

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Abstract
Catharanthus roseus (Alba) is a medicinal plant known for its indole alkaloids, including vincristine and vinblastine. The study focused on comparing different extraction methods and selecting a suitable solvent system for thin layer chromatography to observe alkaloid separation. Five extracts were prepared using crude leaf, callus, cold methanol, root tissue and Soxhlet extraction. All extracts were tested for the presence of alkaloids, followed by TLC analysis. Among the solvent systems examined, the mixture of ethyl acetate, benzene, methanol and 25% ammonia in the ratio 100:5:5:3 gave the most distinct separation. Cold methanolic and Soxhlet extracts produced the highest number of visible spots under UV light at 254 nm. Heat activation of plates altered the visibility of some bands, which highlighted changes in the interaction between the extract and the mobile phase. The method developed in this work offers a simple and inexpensive way to observe alkaloids in *C. roseus* and may be used as a preliminary step before advanced chromatographic techniques.

Highlights
- A TLC method was used to observe Pharmaceutically important alkaloids in *Catharanthus roseus* (Alba).
- The solvent mixture of ethyl acetate, benzene, methanol and ammonia gave the best separation.
- Cold methanolic and Soxhlet extracts produced the highest number of visible spots.
- Heat activation changed the clarity and colour of several bands.
- The method is suitable for preliminary alkaloid screening.

Keywords: Alkaloid profiling, *Catharanthus roseus* (Alba), thin layer chromatography, solvent optimization, methanolic extract, vincristine, vinblastine

1. Introduction
Catharanthus roseus (Alba) is a well-known medicinal plant valued for its indole alkaloids, including vincristine and vinblastine. These compounds are used in the treatment of several cancers, and their presence varies across different parts of the plant. Because of this chemical diversity, the plant has been widely studied for its phytochemical and pharmacological properties. (Goswami *et al*., 2024) [3].

Thin layer chromatography is commonly used as a preliminary method to observe the presence of alkaloids in plant extracts. It is simple to perform and helps in selecting suitable solvent systems before using more advanced chromatographic techniques. However, differences in extraction methods and solvent mixtures can influence the clarity and number of alkaloid spots obtained on TLC plates. Only a few studies have compared extraction techniques together with solvent optimisation for the Alba variety.

The present work focuses on preparing different extracts of *C. roseus* (Alba) and selecting an appropriate TLC solvent system for qualitative alkaloid profiling. The study also examines the effect of heat activation on spot development. The aim is to provide a straightforward and reliable procedure that can serve as a first step before detailed chromatographic analysis.

2. Materials and Methods
2.1 Plant Material
Fresh leaves of *Catharanthus roseus* (Alba) were collected from Chhindwara, Madhya Pradesh. The material was washed with distilled water, shade dried and ground into powder. The powder was stored in an airtight container until use.

2.2 Preparation of Extracts: Five extracts were prepared to compare alkaloid separation.

2.2.1 Crude leaf extract
Seven grams of powdered leaf material was soaked in methanol for 24 hours and filtered.

2.2.2 Callus extract
Callus tissue was crushed in 2 mL methanol, filtered and used directly.

2.2.3 Cold methanolic extract
Powdered leaves were shaken in 100 mL methanol for 48-72 hours. The filtrate was concentrated.

2.2.4 Root extract
Dried roots were powdered, soaked in methanol and filtered.

2.2.5 Soxhlet extract: Twenty grams of leaf powder was extracted using methanol in a Soxhlet unit until the solvent ran clear. The residue was concentrated.

2.3. Thin Layer Chromatography
TLC was performed on silica gel 60 F_{254} plates (0.2 mm thick, Merck). Plates were activated at 100 °C for 30 minutes before use. Each extract (5-10 µL) was applied using a capillary tube.

The chamber was saturated for 20 minutes before development. The solvent mixture used was ethyl acetate, benzene, methanol and 25 percent ammonia in the ratio 100:5:5:3. Plates were dried and observed under UV light at 254 nm. Retention factors were calculated.

Table 1: Solvent Systems Evaluated for TLC Separation

Sr. No.	Solvent System	Ratio	Reference
1	Chloroform: Methanol	8: 2	Kale *et al*., 2018 [6]
2	Ethyl acetate: Benzene: Methanol: 25% Ammonia	100: 5: 5: 3	Magagula *et al*., 2012 [7]
3	Toluene: Methanol: Diethylamine	8: 7.5: 0.75	Barkat *et al*., 2015 [1]
4	Toluene: Acetone: Methanol: Ammonium hydroxide	28: 10: 2: 0.5	Goodbody *et al*., 1989 [2]

3. Results
3.1 Thin Layer Chromatography Profiles of *Catharanthus roseus* (Alba) Extracts

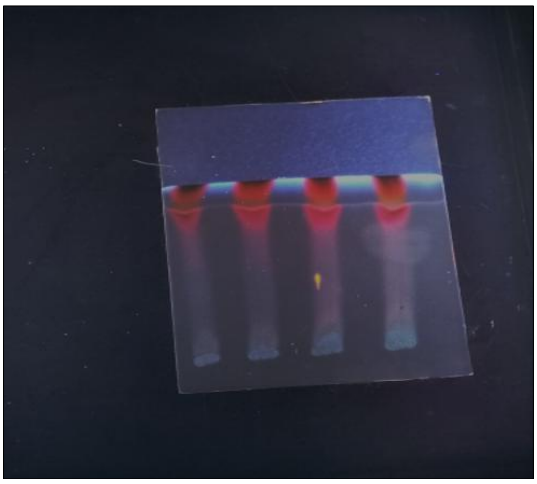

Fig 1: TLC profile of the crude leaf extract showing dark orange-brown and pink bands under UV light

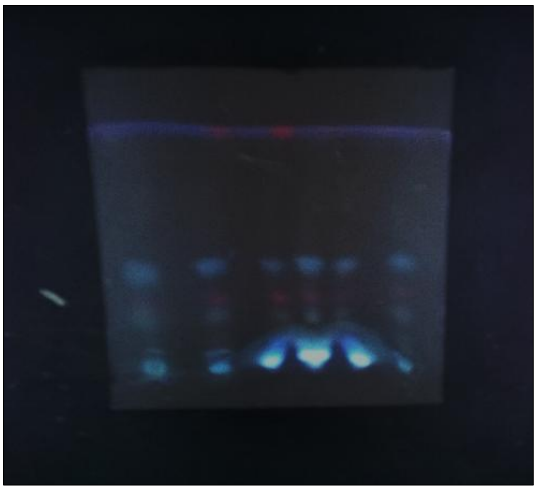

Fig 2: TLC profile of the cold methanolic leaf extract showing multiple alkaloid bands under UV light. (Discuss in Table 2.)

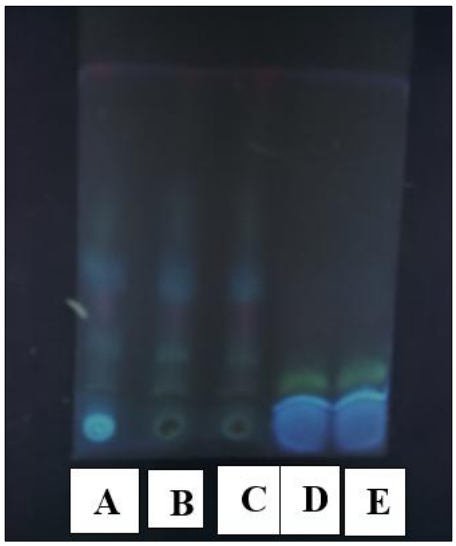

Fig 3: TLC profiles of extracts before heat activation: A, cold methanolic extract; B-C, Soxhlet extract; D-E, callus extract. (Discuss in Table 2.)

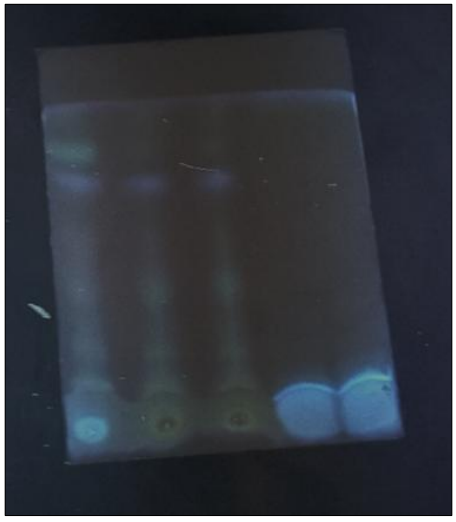

Fig 4: TLC profile of the Soxhlet extract after heat activation showing purple and light-yellow green bands. (Discuss in Table 2.)

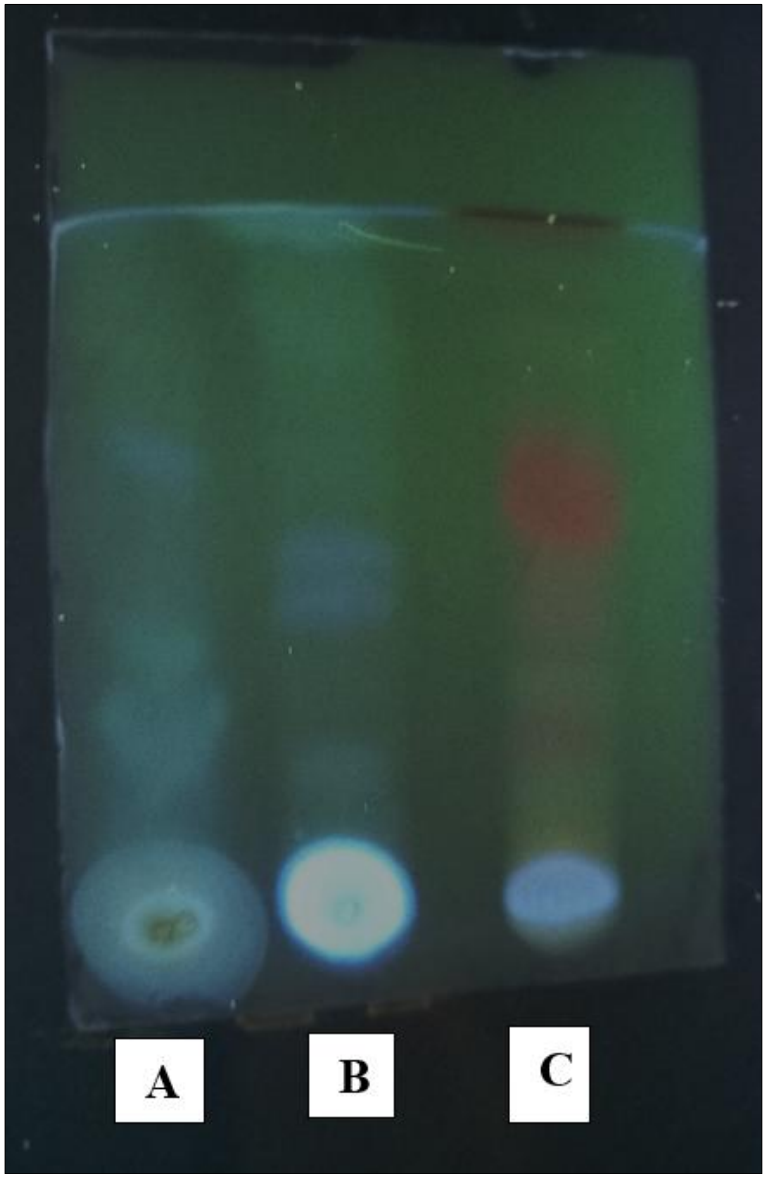

Fig 5: TLC profiles of root and leaf extracts: A, crude root extract; B, sonicated root extract; C, sonicated leaf extract. (Discuss in Table 3.)

Table 2: Rf value of Cold Methanolic and Soxhlet Leaf Extracts of *C. roseus*

Sr. No.	Colour (Cold Methanol)	Colour (After Heat)	Colour (Soxhlet)	Colour (After Heat)	R.F Value
1	Pale yellow green	Light cream-yellow	Pale yellow green	Light cream yellow	0.07
2	Light blue green	Pale blue	Light blue/green	Pale blue	0.14
3	Light pink	Faded	Light pink	Disappeared	0.25
4	Sky blue	Light blue	Sky blue	Light blue	0.33
5	Light cream	Faint cream	Light cream	Faint cream	0.56
6	Not detected	Not detected	Not detected	Purple	0.64
7	Not detected	Not detected	Not detected	Green	0.73
8	Orange	Orange disappeared	Orange	Orange disappeared	0.85

Table 3: Rf Values of Crude Root, Sonicated Root and Crude Leaf Extracts

Spot No.	Crude Root (Rf)	Colour	Sonicated Root (Rf)	Colour	Crude Leaf (Rf)	Colour
1	0.26	Light blue	0.16	Light green	0.10 (unclear)	Light yellow trail
2	0.40	Light green	0.42	Light purple	0.26 (unclear)	Light pink trail
3	0.66	Light purple	0.45	Light purple	0.40	Light yellow
4	0.70	Yellow trail	0.70	Yellow trail	0.60	Dark pink

4. Discussion

Thin layer chromatography was useful for observing alkaloids in *Catharanthus roseus* (Alba). Clear bands appeared under UV light at 254 nm in all extracts, which showed that each sample contained more than one alkaloid. The comparison between cold methanolic, Soxhlet, root, callus and column-derived extracts showed differences in the number of bands and their appearance. This indicates that the method of extraction affects how well alkaloids separate on the TLC plate.

The choice of solvent also influenced the quality of separation. The mixture of ethyl acetate, benzene, methanol

and 25% ammonia in the ratio 100:5:5:3 gave the most stable and clear bands. The bands observed in this system, including shades of green, pink, blue, yellow-green, purple and orange, reflected the movement of compounds with different polarities (shown in fig.2,3,4 and 5).

Extraction conditions played an important role in the clarity of the chromatographic results. The cold methanolic extract produced eight well-defined bands, while the crude extract produced only one visible spot. The Soxhlet extract gave a pattern similar to the cold methanolic extract, which supports the use of methanol as an effective solvent for alkaloid extraction. In this experiment callus extract showed only a faint green band with trailing, suggesting a lower level of alkaloids compared to the plant grown in the field.

Overall, the observations show that the separation of alkaloids depends on both the extraction method and the solvent used for TLC. Alkaloid presence was supported by the TLC patterns. Using reference standards such as vincristine and vinblastine, along with techniques like high-performance thin layer chromatography, would allow for clearer identification and estimation of individual compounds. The method presented in this study can serve as a simple and economical first step for examining alkaloids in *C. roseus* and similar medicinal plants.

5. Conclusion

The developed Thin Layer Chromatography (TLC) method provides a simple, rapid, and cost-effective approach for the qualitative analysis of alkaloids in *Catharanthus roseus* (Alba). Optimization of the solvent system, particularly ethyl acetate: benzene: methanol:25% ammonia (100:5:5:3), resulted in distinct and reproducible chromatographic bands under UV light. Comparative analysis of different extracts demonstrated that cold methanolic and Soxhlet extracts yielded maximum resolution, confirming methanol as an efficient solvent for alkaloid extraction. The study establishes TLC as a reliable preliminary tool for screening and identification of bioactive compounds in *C. roseus*. The components of the callus require an appropriate method for their separation. Future work will focus on addressing this aspect. The method can be further validated using HPTLC for quantitative estimation and applied in quality control and standardization of *C. roseus*-based herbal formulations.

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