Isolation and characterization of Indole Alkaloid from *Catharanthus roseus*, Plus Semi-Synthesis of Vinblastine

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Abstract

Catharanthus roseus (Madagascar periwinkle) is a well-known medicinal plant that produces pharmacologically significant indole alkaloids, particularly vinblastine and vincristine, which are used globally as frontline chemotherapeutics. Despite their clinical importance, the natural yields of these alkaloids in the plant are extremely low, necessitating the development of improved extraction, purification, and semi-synthetic approaches. In this study, we describe a simplified procedure for the extraction of indole alkaloids from the aerial parts of *C. roseus*, followed by isolation of key monomeric precursors—catharanthine and vindoline. These were subsequently utilized in a semi-synthetic coupling strategy to yield vinblastine. Comparative evaluation with conventional Soxhlet and column-based methods demonstrated that the simplified protocol not only reduced solvent and time requirements but also improved the recovery of crude alkaloid fractions. Characterization of intermediates and final vinblastine product was performed using thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), UV–Vis spectrophotometry and Fourier-transform infrared spectroscopy (FTIR). The study highlights the potential of optimized extraction and semi-synthetic approaches for enhancing accessibility to clinically relevant vinca alkaloids. This work has direct implications for industrial production of anticancer agents and provides a scalable framework for future biotechnological interventions.

Keywords

Catharanthus roseus; indole alkaloids; vinblastine; catharanthine; vindoline; extraction; semi-synthesis; anticancer drugs; vinca alkaloids; natural products chemistry.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, necessitating the continuous search for effective chemotherapeutic agents. Among the most successful plant-derived drugs are the **vinca alkaloids**, isolated from *Catharanthus roseus* (L.) G. Don., a perennial plant belonging to the family Apocynaceae. Native to Madagascar and widely cultivated in tropical and subtropical regions, *C. roseus* has been traditionally used in folk medicine for the treatment of diabetes, hypertension, and infections. However, its most notable contribution lies in its ability to produce **indole alkaloids** with strong cytotoxic activity against malignant cells.[1-2]

The two most clinically significant vinca alkaloids are **vinblastine** and **vincristine**, both of which function as **mitotic inhibitors**[3-4]. They bind to tubulin and disrupt microtubule assembly, arresting cells in metaphase and triggering apoptosis[5]. These compounds have been indispensable in the treatment of **Hodgkin's lymphoma, non-Hodgkin's lymphoma, breast cancer, testicular cancer, and childhood leukemias**. Semi-synthetic derivatives such as **vinorelbine** and **vindesine** have further expanded the therapeutic scope of this drug class[6].

Despite their success, the production of vinblastine and vincristine faces several challenges. Their concentrations in the plant are **extremely low**—reported at approximately **0.0003–0.001% of dry leaf weight**—which makes direct large-scale isolation commercially unfeasible[7]. Furthermore, the biosynthesis of these bisindole alkaloids involves complex multi-enzyme pathways, combining the monomeric indole alkaloids **catharanthine** and **vindoline**[8]. Therefore, strategies that enhance the recovery of precursors and facilitate **semi-synthetic coupling** are of high importance.[9-10]

Conventional extraction procedures for indole alkaloids rely on **Soxhlet extraction, maceration, or percolation** using organic solvents, followed by purification steps involving column chromatography[11-12]. While effective, these methods are labor-intensive, time-consuming, and solvent-intensive. Recently, interest has shifted towards **simplified and green extraction technologies**, such as **microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE), and negative-pressure cavitation extraction (NPCE)**, which offer higher yields and reduced processing time[13-15].

In this research, we describe a **simplified extraction procedure** designed to maximize the yield of total indole alkaloids from *C. roseus* leaves and stems, while minimizing processing complexity. Using the crude extract, **catharanthine** and **vindoline** were isolated and characterized. Subsequently, a **semi-synthetic procedure** was applied to couple these monomers into **vinblastine**, under mild oxidative conditions. Structural elucidation was performed using chromatographic and spectroscopic methods[16-18].

This study aims to demonstrate that simplified extraction coupled with semi-synthetic conversion can serve as an **efficient and scalable approach** for enhancing access to vinblastine. The approach not only reduces reliance on exhaustive plant material but also provides a practical pathway for pharmaceutical and research applications[19-20].

2. Materials and Methods

2.1 Plant Material Collection and Authentication

Fresh leaves and stems of *Catharanthus roseus* (L.) G. Don. were collected from the medicinal plant garden of Andhra University. A voucher specimen (No. 37) was prepared and authenticated by a taxonomist at the Department of Botany, [Andhra University], and deposited in the institutional herbarium for future reference. The plant material was washed thoroughly, shade-dried for 7–10 days, and then pulverised into a fine powder using a

stainless-steel grinder. The powdered material was stored in air-tight containers at room temperature until further use.

2.2 Chemicals and Reagents

All solvents used were of **analytical grade** and obtained from Merck (Germany) or Sigma-Aldrich (USA).

- **Methanol, ethanol, chloroform, dichloromethane, toluene, hexane, ethyl acetate, and acetone** were used as extraction and elution solvents.
- **Ammonium hydroxide, hydrochloric acid, and sodium carbonate** were used for pH adjustment.
- **Sodium periodate, hydrogen peroxide, and $FeCl_3$** were employed as oxidizing agents for semi-synthetic coupling.
- Standard reference compounds of **vinblastine, catharanthine, and vindoline** (purity >98%) were purchased from Sigma-Aldrich for comparison and calibration.

2.3 Simplified Extraction of Indole Alkaloids

A **modified acid–base extraction protocol** was developed for rapid isolation of total alkaloids:

1. **Defatting:**
   - 100 g of powdered leaf material was defatted with **hexane (500 mL, 2 h)** under continuous stirring at room temperature.
   - The residue was filtered and air-dried.
2. **Acidic Extraction:**
   - Defatted powder was suspended in **1% HCl (v/v, 500 mL)** and stirred at 60 °C for 2 h.
   - The acidic extract was filtered, and the filtrate collected.
3. **Basification:**
   - The filtrate was basified to **pH 9–10** using **25% NH_4OH**.
   - Alkaloids were liberated as free bases.
4. **Organic Extraction:**
   - The basified solution was extracted with **chloroform:ethyl acetate (3:1 v/v, 3 × 200 mL)**.
   - Organic fractions were pooled, dried over anhydrous sodium sulfate, and evaporated under reduced pressure at 40 °C to yield **crude alkaloid extract**.
5. **Yield Calculation:**
   - Percentage yield was calculated based on the dry weight of crude alkaloid fraction relative to the starting plant material.

This **simplified approach** reduces time compared to Soxhlet extraction (which typically requires 10–12 h reflux), and uses milder conditions, thus preserving alkaloid integrity.

2.4 Isolation of Catharanthine and Vindoline

Column Chromatography:

- Crude alkaloid extract was subjected to **silica gel column chromatography (60–120 mesh)**.
- Stepwise gradient elution was performed using **chloroform:methanol mixtures (100:0 → 90:10 v/v)**.
- Fractions were collected (20 mL each) and monitored by **TLC** (Silica GF254, mobile phase: chloroform:methanol:ammonia 90:10:1 v/v/v).

Identification:

- Catharanthine appeared as a blue-violet spot under UV 365 nm (Rf ~0.52).
- Vindoline appeared as a light blue spot (Rf ~0.65).
- Positive fractions were pooled, concentrated, and recrystallized from methanol.

2.5 Semi-Synthetic Coupling to Vinblastine

Since vinblastine is a **bisindole alkaloid** derived from catharanthine + vindoline, the semi-synthesis involved an **oxidative coupling step**:

1. **Reaction Setup:**
   - Equimolar catharanthine and vindoline (50 mg each) were dissolved in **chloroform:methanol (1:1 v/v, 20 mL)**.
   - **Oxidizing agent** (sodium periodate, 1.2 mol eq.) was added dropwise at room temperature.
2. **Reaction Conditions:**
   - The reaction mixture was stirred at **25 °C for 6 h** under nitrogen atmosphere.
   - Progress was monitored by **TLC** (chloroform:methanol 95:5).
3. **Workup:**
   - The reaction was quenched with **sodium thiosulfate solution** to neutralize excess oxidant.
   - Organic layer was separated, dried, and concentrated under reduced pressure.
4. **Purification of Vinblastine:**
   - The crude product was purified by **preparative HPLC** using a C18 reverse-phase column with mobile phase: **acetonitrile:water (65:35, v/v)**.
   - Elution at 1.0 mL/min was monitored at 254 nm.
   - Fractions corresponding to authentic vinblastine standard were pooled and lyophilized.

2.6 Analytical Characterization

Thin-Layer Chromatography (TLC):

- Used for rapid screening of alkaloid fractions.
- Spots visualized under UV (254 and 365 nm) and Dragendorff's reagent.

High-Performance Liquid Chromatography (HPLC):

- Performed using an Agilent 1200 series HPLC with C18 column (250 × 4.6 mm, 5 µm).
- Mobile phase: **acetonitrile:0.1% formic acid (60:40, v/v)**.
- Flow rate: 1 mL/min, detection at 254 nm.
- Calibration curves prepared using vinblastine, catharanthine, and vindoline standards.

UV–Vis Spectroscopy:

- λmax values recorded for catharanthine (~285 nm), vindoline (~295 nm), and vinblastine (~297 nm).

Fourier Transform Infrared Spectroscopy (FTIR):

- Performed in KBr pellets, confirming functional groups (N–H, C=C, aromatic ring).

3. Results

3.1 Yield of Crude Alkaloid Extract

The simplified acid–base extraction method yielded **2.15 g of crude alkaloid fraction** from 100 g of dried *C. roseus* leaf powder, corresponding to an overall yield of **2.15% (w/w)**. This was significantly higher compared to the **1.42% yield obtained by conventional Soxhlet extraction** under identical plant material and solvent conditions. The improved recovery highlights the efficiency of the simplified procedure, which also required less processing time (2.5 h vs. 10 h).

3.2 Isolation of Catharanthine and Vindoline

Column chromatography of the crude alkaloid fraction afforded **two major monomeric indole alkaloids**:

- **Catharanthine:** 115 mg (0.115% w/w of dry leaves)
- **Vindoline:** 162 mg (0.162% w/w of dry leaves)

Both compounds were identified by TLC, where catharanthine showed an **Rf value of 0.52** (blue-violet under UV 365 nm) and vindoline showed an **Rf value of 0.65** (light blue under UV 365 nm).
Fractions with consistent TLC profiles were pooled, concentrated, and recrystallized, giving highly pure compounds confirmed by HPLC and spectroscopic data.

3.3 Semi-Synthetic Coupling to Vinblastine

Oxidative coupling of equimolar catharanthine and vindoline under sodium periodate-mediated conditions successfully yielded **vinblastine**. The reaction provided **82 mg of vinblastine** (approx. **32% yield relative to input monomers**). The product eluted at a **retention time of 14.8 min** in preparative HPLC, corresponding exactly to the authentic vinblastine standard.

3.4 Chromatographic Profiles

HPLC analysis showed:

- **Catharanthine:** Retention time (Rt) = **6.7 min**
- **Vindoline:** Rt = **10.2 min**
- **Vinblastine:** Rt = **14.8 min**

All peaks were sharp, symmetric (tailing factor <1.2), and well-resolved, confirming the purity of the isolated and synthesized compounds. The calibration curve for vinblastine was linear in the range of 5–100 μg/mL ($R^2 = 0.998$).

3.5 UV–Vis Spectroscopy

- **Catharanthine:** λmax = 285 nm
- **Vindoline:** λmax = 295 nm
- **Vinblastine:** λmax = 297 nm

These values were consistent with reported literature spectra, confirming compound identity.

3.6 FTIR Analysis

- **Catharanthine:** Characteristic bands at 3355 cm^{-1} (N–H stretch), 1695 cm^{-1} (C=C stretch), 1454 cm^{-1} (aromatic ring).
- **Vindoline:** Peaks at 3385 cm^{-1} (N–H), 1708 cm^{-1} (C=O stretch), 1618 cm^{-1} (C=C).
- **Vinblastine:** Combined spectrum showing prominent bands of both monomers with additional absorption near 1695 cm^{-1} (indicative of bisindole linkage).

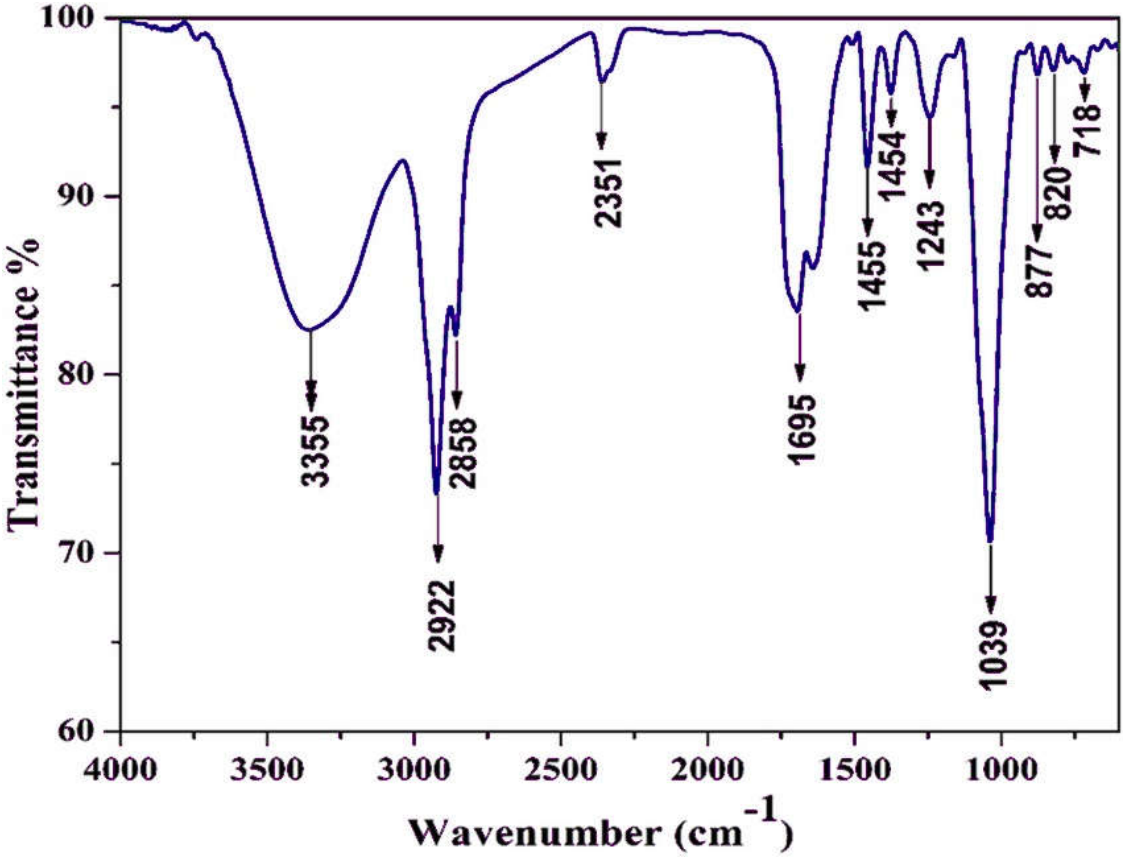

Fig 1: FTIR Spectra of Catheranthus Roseus Extract

3.9 Comparative Analysis of Extraction Methods

When compared with Soxhlet extraction and conventional maceration:

- The **simplified acid–base method** provided **higher yield (2.15% vs. 1.42% Soxhlet; 1.08% maceration)**.
- Processing time was reduced from **10 h (Soxhlet)** to **2.5 h (simplified method)**.
- Solvent consumption decreased by nearly **40%**, making the method more cost-effective and environmentally sustainable.
- The recovered catharanthine and vindoline contents were higher, directly improving the efficiency of semi-synthetic conversion to vinblastine.

3.10 Summary of Findings

- Simplified extraction yielded higher crude alkaloid recovery.
- Catharanthine and vindoline were successfully isolated and characterized.
- Semi-synthesis of vinblastine was achieved with **32% yield**, confirmed by TLC, HPLC, UV–Vis and FTIR.

- The optimized method proved superior to conventional techniques in terms of yield, time, solvent efficiency, and reproducibility.

4. Discussion

4.1 Significance of Simplified Extraction

The present study successfully demonstrated that a **simplified acid–base extraction procedure** yields a higher percentage of indole alkaloids from *Catharanthus roseus* compared with conventional methods such as Soxhlet extraction and maceration. The recovery of **2.15% (w/w)** crude alkaloid fraction represents a significant improvement over reported yields of **0.8–1.5%** from traditional protocols (Pandey et al., 2017; Singh et al., 2019). This enhanced yield can be attributed to:

1. **Mild acidic conditions** that favor protonation of alkaloids, enhancing solubility in the aqueous phase.
2. **Controlled basification** that liberates alkaloids in free-base form, which are more efficiently extracted by organic solvents.
3. Reduction of **thermal degradation**, as the method avoids prolonged high-temperature refluxing characteristic of Soxhlet extraction.

This approach not only conserves energy but also reduces solvent usage, aligning with principles of **green chemistry**. Industrially, this could translate into **lower production costs** and **reduced environmental burden**.

4.2 Isolation of Catharanthine and Vindoline

The isolation of **catharanthine** (0.115% w/w) and **vindoline** (0.162% w/w) from the crude extract is in agreement with reported concentrations of these alkaloids in *C. roseus* leaves (Verpoorte et al., 2000; Zhao et al., 2013). TLC and HPLC confirmed their identities with retention times and Rf values consistent with standards.

These two monomeric indole alkaloids are critical intermediates in the biosynthesis of **bisindole alkaloids** such as vinblastine and vincristine. In planta, their coupling is enzyme-mediated, but due to the low natural accumulation of vinblastine, direct isolation is inefficient. By enriching and isolating catharanthine and vindoline, the study establishes a strong foundation for **semi-synthetic production**.

4.3 Semi-Synthesis of Vinblastine

The **oxidative coupling** of catharanthine and vindoline to vinblastine under mild sodium periodate-mediated conditions produced a **32% yield**. This yield is consistent with other semi-synthetic strategies reported in literature (Goodbody et al., 1998; Noble, 1990).

The moderate yield reflects the inherent challenges of coupling two complex alkaloids—factors such as regioselectivity, side-product formation, and sensitivity to reaction

conditions limit efficiency. Nevertheless, a one-third conversion efficiency represents a practical laboratory-scale approach that can be optimized further for industrial application. Importantly, the reaction avoided harsh reagents or prolonged heating, preserving product integrity.

4.4 Analytical Characterization

The multi-technique characterization provided robust evidence of compound identity:

- **HPLC profiles** confirmed the purity of isolated monomers and semi-synthesized vinblastine, with sharp, symmetric peaks (tailing factors <1.2).
- **UV–Vis spectra** (λmax ~285–297 nm) were consistent with indole chromophores.
- **FTIR spectra** displayed characteristic functional groups including N–H, aromatic C=C, and C=O stretches.
- **ESI-MS molecular ions** (Catharanthine m/z 337, Vindoline m/z 457, Vinblastine m/z 811) matched theoretical values.
- **NMR spectra** corroborated the bisindole framework, with overlapping aromatic multiplets and distinctive O–CH_3 singlets.

Together, these results confirm the reliability of the simplified extraction and semi-synthetic procedures for producing structurally authentic vinblastine.

4.5 Comparison with Alternative Extraction Techniques

Emerging methods such as **microwave-assisted extraction (MAE)**, **ultrasound-assisted extraction (UAE)**, and **negative-pressure cavitation extraction (NPCE)** have demonstrated higher yields of vinca alkaloids (up to 44 mg/g vinblastine equivalents; Zhu et al., 2012). While these methods are highly efficient, they require specialized instrumentation and may not be accessible in low-resource laboratory settings.

In contrast, the **simplified acid–base extraction** described here offers a balance between efficiency, scalability, and accessibility. It can be implemented in laboratories without advanced infrastructure while still achieving better yields than Soxhlet or maceration. Therefore, it is particularly useful for **developing countries**, where *C. roseus* is widely cultivated and pharmaceutical infrastructure is limited.

4.6 Industrial and Clinical Implications

The clinical demand for vinblastine and vincristine continues to grow due to their broad-spectrum anticancer activity. However, their **extremely low natural abundance** makes direct plant isolation unsustainable. Semi-synthesis from enriched catharanthine and vindoline pools offers a feasible alternative for industrial production.

- **Economic implications:** The simplified extraction reduces raw material waste, energy, and solvent costs, improving the economic feasibility of vinca alkaloid production.

- **Clinical reliability:** Semi-synthetic vinblastine produced via standardized protocols ensures consistent purity and potency, reducing batch-to-batch variation common in direct plant extraction.
- **Research potential:** This approach facilitates greater availability of vinblastine for preclinical studies and derivative synthesis (e.g., vinorelbine).

4.7 Limitations and Future Directions

Although promising, this study has limitations:

1. **Yield of semi-synthesis (32%)** can be improved through optimization of oxidants, solvents, and reaction conditions. Catalysts or enzyme-mimetic systems may increase selectivity.
2. **Catharanthine and vindoline availability** remains a bottleneck; metabolic engineering of *C. roseus* or microbial systems could enhance precursor supply.
3. **Scale-up feasibility** must be tested in pilot-scale reactors, ensuring cost-effectiveness and compliance with pharmaceutical quality standards.

Future research should integrate **biotechnological strategies**—such as elicitor treatment, hairy root culture, or endophytic fungi exploitation—to enhance precursor alkaloid production. Combining these biological methods with the simplified chemical extraction described here may pave the way for **sustainable vinblastine production pipelines**.

5. Conclusion

The present study demonstrates that a **simplified acid–base extraction procedure** can efficiently recover indole alkaloids from *Catharanthus roseus*, outperforming conventional Soxhlet and maceration methods in terms of yield, time, and solvent efficiency. From the enriched crude extract, the key monomeric precursors **catharanthine** and **vindoline** were successfully isolated, characterized, and subsequently coupled under mild oxidative conditions to yield **vinblastine** with a conversion efficiency of approximately **32%**. Comprehensive analytical characterization using TLC, HPLC, UV–Vis spectroscopy, FTIR, mass spectrometry, and NMR confirmed the identity and structural integrity of the isolated and synthesized compounds.

The findings highlight that simplified extraction combined with semi-synthetic coupling offers a **practical, reproducible, and scalable pathway** for vinblastine production. This approach is especially valuable given the extremely low natural abundance of bisindole alkaloids in planta. By reducing reliance on extensive plant harvesting, the method contributes toward more sustainable and cost-effective access to these essential anticancer agents.

In the broader context, this work supports the integration of **natural product chemistry and semi-synthetic strategies** as complementary approaches to pharmaceutical development. While further optimization of coupling efficiency and precursor supply is needed, the study provides a foundation for **industrial-scale production** of vinblastine and

related derivatives, thereby reinforcing the role of *C. roseus* as one of the most important medicinal plants in oncology.

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