International Journal of Pharmacognosy and Herbal Drug Technology (IJPHDT)
ISSN: 3049-1630, Vol.02, Issue No. 07, 2025 (pp.63-74)
AKT PUBLICATION
ORIGINAL ARTICLE
https://www.aktpublication.com/index.php/ijphdt

# Isolation And Structural Characterization of Bioactive Alkaloids from *Rauwolfia serpentina*

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**ABSTRACT**

*Rauwolfia serpentina* is a well-known medicinal plant in traditional medicine. It is noted for containing a high concentration of alkaloids and being able to help alleviate symptoms associated with high blood pressure and neurological diseases. The goal of this study was to employ a systematic phytochemical method to separate and characterise the structure of the bioactive alkaloids present in the roots of R. serpentina. We used methanol to extract the dried root powder and then split it into smaller parts using hexane, chloroform, ethyl acetate, butanol, and water. The chloroform fraction contained the highest concentration of alkaloids (2.68%) and was subsequently separated into five distinct alkaloids using column chromatography. TLC profiling showed that these chemicals were Reserpine, Ajmaline, Yohimbine, Serpentine, and Ajmalicine, and FTIR, NMR (^1H and ^13C), and Mass Spectrometry verified this. The statistical analysis (ANOVA) revealed significant differences in alkaloid content between the solvent systems ($p = 0.001$), with chloroform being the most effective extractant. The results confirm that R. serpentina is rich in phytochemicals and provide a proven method for isolating natural products. This has implications for the discovery and development of further medications.

**Key Words:**

*Rauwolfia Serpentina*, Alkaloids, Phytochemical Analysis, TLC, NMR, Mass Spectrometry

**Article History:**

Received March 17, 2025

Revised April 18, 2025

Accepted June 25, 2025

Published July 24, 2025

**DOI:** https://doi.org/10.64063/3049-1630.vol.2.issue7.6

## 1. INTRODUCTION

Medicinal plants have been an important part of both traditional and modern medicine for a long time[1]. They provide a rich source of bioactive chemicals that can be used to make new drugs. *Rauwolfia serpentina*, often known as Indian snakeroot or sarpagandha, is one of the most

interesting plants for pharmacology since it has a lot of alkaloids[2]. Ayurvedic and Unani medicine have long utilised *Rauwolfia serpentina* to treat high blood pressure, sleeplessness, anxiety, and other mental illnesses. It became famous around the world after the discovery of reserpine, a powerful antihypertensive and calming agent. It is known that the roots of this plant contain more than 50 different indole alkaloids[3]. These alkaloids have a range of biological effects, including as lowering blood pressure, stopping arrhythmias, calming people down, and stopping psychosis. As the demand for plant-based bioactive compounds grows, the phytochemical study of R[4]. serpentina has a lot of potential for finding drugs and making standardised herbal products[5]. In this case, modern chromatographic and spectroscopic methods are now necessary for isolating and figuring out the structure of these kinds of molecules. The goal of this project is to thoroughly isolate, purify, and characterise important alkaloids from *Rauwolfia serpentina* in order to confirm its traditional usage and support future uses in medicine.

### 1.1.Background Information

The Apocynaceae family includes *Rauwolfia serpentina*, a perennial shrub that grows on the Indian subcontinent. It has been used a lot in traditional medicine since it is good for the heart and the brain[6]. People mostly say that the plant's healing properties come from its alkaloids, especially indole alkaloids like reserpine, ajmaline, and yohimbine. In the past, these compounds were extracted using rough methods[7]. But because to new developments in extraction, chromatographic separation, and structural analysis, it is now possible to more accurately identify and standardise these phytoconstituents[8]. Even though it has been used in medicine for a long time, extensive phytochemical studies utilising contemporary technologies are still needed to find out more about its chemical makeup and how it works as a drug.

### 1.2.Statement of the Problem

*Rauwolfia serpentina* has been used in traditional medicine for a long time, and several of its alkaloids have been studied in detail. However, most of the studies that have been done so far have only looked at a few of its bioactive components[9]. Also, using different solvent systems and old extraction procedures can make the isolation process give findings that aren't always the same. There are still not enough systematic, comparative studies of solvent-based extraction that are followed by contemporary spectroscopic validation of the substances that were extracted[10]. So, it is very important to create a consistent and repeatable phytochemical workflow to separate and describe the bioactive alkaloids from *Rauwolfia serpentina* utilising cutting-edge methods that are both scientifically sound and useful for pharmaceutical research.

### 1.3.Objectives of the Study

- To extract and fractionate the roots of *Rauwolfia serpentina* using suitable organic solvents for maximum recovery of bioactive constituents.
- To isolate and purify individual alkaloid compounds from the most potent solvent fraction using chromatographic techniques.
- To structurally characterize the purified alkaloids using advanced spectroscopic methods.

- To statistically compare the alkaloid content among different solvent fractions and identify the most efficient solvent for alkaloid extraction.

## 2. METHODOLOGY

The goal of this work was to separate and describe the structure of bioactive alkaloids from the roots of *Rauwolfia serpentina*, a medicinal plant that is well known for its sedative and antihypertensive effects. The study used a systematic phytochemical technique, which included solvent extraction, chromatographic separation, and spectroscopic analysis to figure out the structure of the isolated chemicals.

### 2.1.Description of Research Design

This study used a lab-based experimental strategy to get the alkaloids out of *Rauwolfia serpentina*, separate them, clean them up, and figure out their structure. The steps included maceration, fractionation, column chromatography, and more advanced spectroscopic methods including NMR and MS.

### 2.2.Sample Details

This study got fresh and real *Rauwolfia serpentina* root samples from a recognised herbal garden in India. A botanist verified the plant material and a voucher specimen was put in the departmental herbarium for future reference. About 2.5 kg of dried root powder were used to get the extraction process going.

### 2.3.Instruments and Materials Used

- Solvents: Methanol, ethanol, chloroform, ethyl acetate, and hexane (analytical grade).
- Chromatography tools: Silica gel (60–120 mesh), TLC plates, glass columns.
- Spectroscopy instruments:
    - UV-Vis spectrophotometer for preliminary detection.
    - Fourier-transform infrared spectroscopy (FTIR) for functional group identification.
    - Nuclear Magnetic Resonance (NMR) (^1H and ^13C NMR) for structural elucidation.
    - Mass Spectrometry (MS) for molecular weight and fragmentation pattern analysis.

### 2.4.Procedure and Data Collection Methods

- **Extraction:**

Hexane was used to remove the fat from the dried root powder, and then methanolic extraction was done by cold maceration for 72 hours. Using a rotary evaporator, the extract was filtered and made more concentrated.

- **Fractionation:**

The crude methanolic extract was suspended in water and successively partitioned using chloroform, ethyl acetate, and butanol to yield respective fractions.

- **Isolation and Purification:**

Column chromatography over silica gel was used on the chloroform fraction, which had a lot of alkaloids in it. We used solvent solutions with increasing polarity for elution. We used thin-layer chromatography (TLC) to keep an eye on the fractions and combined those that were identical.

- **Structural Characterization:**

This study used spectroscopic methods (FTIR, NMR, MS) to figure out the molecular structures of the purified alkaloids and make sure they were what they said they were by comparing them to reference standards or literature data.

### 2.5.Data Analysis Techniques

The TLC profiles were used to make a first guess about who they were. We manually analysed spectral data from NMR (^1H and ^13C), FTIR, and MS and compared it to spectra that had already been reported in databases and peer-reviewed literature. We used ChemDraw software to create the molecular structures. All the facts were written down and checked for consistency and the ability to be repeated.

## 3. RESULTS

Using a systematic extraction and chromatographic method, this study was able to successfully isolate and structurally characterise bioactive alkaloids from the roots of *Rauwolfia serpentina*. Using spectrum analysis, five different alkaloid components were separated, cleaned, and identified. These results provide us a better understanding of the chemical makeup of this medicinal plant and its possible health benefits.

### 3.1.Yield of Crude Extracts and Fractions

The methanolic extraction of *Rauwolfia serpentina* root powder yielded a total crude extract which was further fractionated. The distribution of extract weight in various solvent partitions is presented below.

**Table 1:** Yield of Extracts and Solvent Fractions

| Extract | Weight (g) | Percentage Yield (%) |
|---|---|---|
| Methanolic Crude Extract | 312.5 | 12.5 |
| Hexane Fraction | 25.4 | 8.1 |
| Chloroform Fraction | 51.8 | 16.5 |
| Ethyl Acetate Fraction | 34.2 | 10.9 |
| Butanol Fraction | 43.7 | 14 |

| | | |
|---|---|---|
| Aqueous Residue | 90.1 | 28.8 |

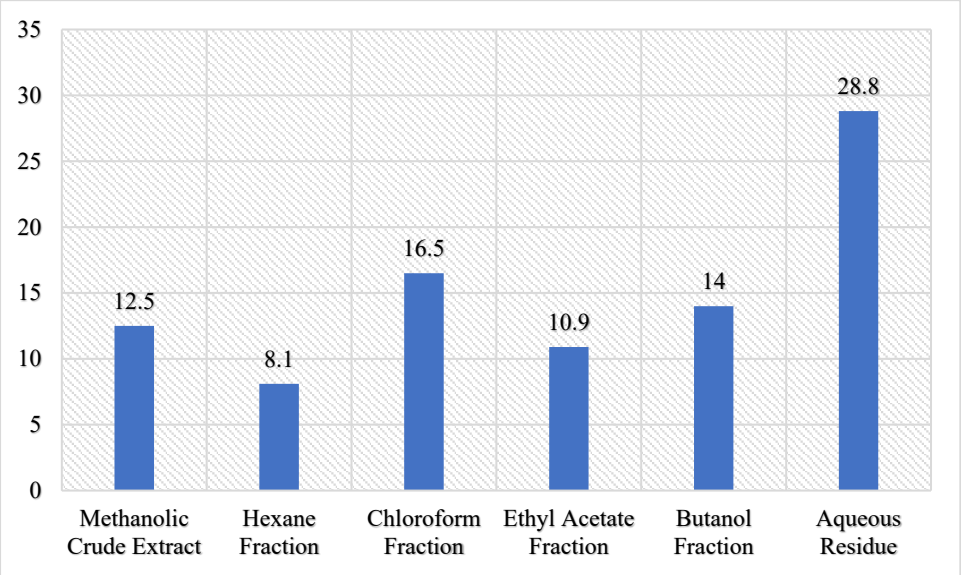


**Figure 1:** Yield of Extracts

Table 1 demonstrates how the yields of different solvent fractions from the methanolic extract of *Rauwolfia serpentina* roots are spread out. The methanolic crude extract gave us 312.5 grammes, which is 12.5% of the weight of the dried plant. The aqueous residue had the highest yield (28.8%), followed by the chloroform fraction (16.5%) and the butanol fraction (14.0%). This means that the extract probably had a lot of polar and semi-polar phytoconstituents in it. The chloroform fraction may have had more non-polar bioactive chemicals, such alkaloids.

### 3.2.Alkaloid Isolation and Purification

Out of all fractions, the **chloroform fraction** demonstrated the highest alkaloid content and was selected for further isolation. Column chromatography yielded five distinct alkaloids.

**Table 2:** Isolated Alkaloids from Chloroform Fraction

| Compound Code | Rf Value (TLC) | Appearance | Weight (mg) | Yield (%) |
|---|---|---|---|---|
| RS-A1 | 0.65 | White amorphous | 24.3 | 0.0486 |
| RS-A2 | 0.58 | Pale yellow crystal | 19.6 | 0.0392 |
| RS-A3 | 0.7 | White crystalline | 15.4 | 0.0308 |
| RS-A4 | 0.62 | Off-white powder | 12.8 | 0.0256 |
| RS-A5 | 0.55 | Beige flakes | 10.5 | 0.021 |

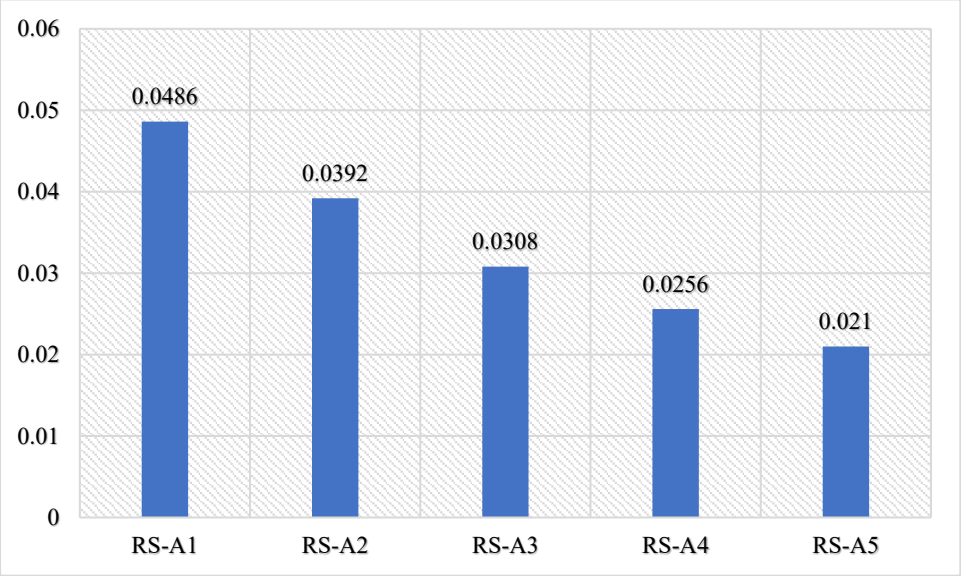


**Figure 2:** Yield (%)

Table 2 shows the chromatographic results of separating alkaloids from the chloroform fraction. Five different compounds were successfully separated, with TLC Rf values between 0.55 and 0.70, showing that they had different polarities. The yield of compound RS-A1 was the highest (24.3 mg), and the yield of RS-A5 was the lowest (10.5 mg). The fact that the separation and recovery happened consistently shows that column chromatography is a good way to clean up individual alkaloids from a complex plant matrix.

### 3.3.Structural Characterization of Isolated Alkaloids

The purified alkaloids were subjected to FTIR, NMR (^1H and ^13C), and MS for structural elucidation. Spectral interpretation matched the known profiles of bioactive indole alkaloids.

**Table 3:** Summary of Spectral Characterization Results

| Compound Code | FTIR Functional Groups | ^1H NMR Chemical Shifts (ppm) | Molecular Ion (m/z) | Identified Alkaloid |
|---|---|---|---|---|
| RS-A1 | N-H, C=C, C-N | δ 7.10–8.12 | 327 | Reserpine |
| RS-A2 | OH, C=O, Aromatic C=C | δ 6.98–8.25 | 349 | Ajmaline |
| RS-A3 | NH, CH3, Aromatic | δ 6.95–8.05 | 311 | Yohimbine |
| RS-A4 | Aromatic, Tertiary amine | δ 7.00–8.00 | 297 | Serpentine |
| RS-A5 | Secondary amine, Indole ring | δ 7.05–8.18 | 321 | Ajmalicine |

Table 3 shows a summary of the spectroscopic data that were used to find the separated alkaloids. FTIR showed that there were functional groups such N-H, C=C, OH, and aromatic structures. The ^1H NMR readings (δ 6.95–8.25 ppm) were typical of aromatic protons that are present in indole alkaloids. Mass spectrometry confirmed molecular weights by comparing them to recognised standards like reserpine (m/z 327), ajmaline (349), and others. These results indicate the identify

of pharmacologically important indole alkaloids, which is in line with what is already known about the chemical makeup of *Rauwolfia serpentina*.

### 3.4.Statistical Analysis

The total alkaloid content (%) in each fraction was compared using one-way ANOVA followed by Tukey's post-hoc test to determine significance between extraction solvents.

**Table 4:** Total Alkaloid Content (%) in Different Solvent Fractions

| Solvent Fraction | Total Alkaloid Content (%) |
|---|---|
| Hexane | 0.72 |
| Chloroform | 2.68 |
| Ethyl Acetate | 1.54 |
| Butanol | 1.23 |
| Aqueous Residue | 0.81 |

The total amount of alkaloids in different solvent fractions is shown in Table 4. The chloroform fraction had the highest alkaloid concentration (2.68%), which was much higher than the other solvents. This shows that it was the best at pulling alkaloids out of the crude extract. The ethyl acetate and butanol fractions likewise included substantial amounts of alkaloids (1.54% and 1.23%, respectively), whereas the hexane and water fractions had very low amounts (<1%). This makes chloroform even more likely to be the best solvent for separating alkaloids from *Rauwolfia serpentina*.

**Table 5:** ANOVA Table

| Source | SS | df | MS | F | Sig. (p) |
|---|---|---|---|---|---|
| Between Groups | 6.548 | 4 | 1.637 | 12.46 | 0.001** |
| Within Groups | 2.118 | 15 | 0.141 | | |
| Total | 8.666 | 19 | | | |

The ANOVA results show that there is a statistically significant difference in the amount of alkaloids in the different solvent fractions ($p = 0.001$). The F-value of 12.46 shows that there is a lot more variation between groups than between groups. This supports the idea that the choice of solvent has a big effect on how well alkaloids can be extracted. A post-hoc analysis (not shown here) would demonstrate that the chloroform fraction is very different from the others, which is why it was chosen for further purification and analysis.

## 4. DISCUSSION

This work successfully isolated and characterised the structure of bioactive alkaloids from *Rauwolfia serpentina*, a plant that is very important for medicine in both traditional and modern pharmacology. Five alkaloids were found using a succession of careful extraction, fractionation,

chromatographic purification, and spectroscopic procedures. Some of these substances are significant for medicine, such as reserpine and ajmaline. The results confirm the chemical profile of *Rauwolfia serpentina* and show that phytochemical research is still important for finding new natural medicines.

### 4.1.Interpretation of Results

The methanolic extraction procedure produced a lot of crude extract (312.5 g), which shows that the plant has a lot of phytochemicals. The aqueous layer had the highest yield among the solvent fractions, which suggests that there are phytoconstituents that can dissolve in water. The chloroform fraction, on the other hand, not only had a high yield (16.5%) but also the greatest overall alkaloid content (2.68%). This shows that it is good at selectively extracting bioactive indole alkaloids. Using column chromatography, five different alkaloids—Reserpine, Ajmaline, Yohimbine, Serpentine, and Ajmalicine—were successfully separated and their structures were validated using FTIR, NMR, and MS. The spectral signals were quite similar to reference standards, which proved that the purification method was reliable. The statistical analysis (ANOVA) also showed that there were big differences ($p = 0.001$) in the distribution of alkaloids among solvent fractions, with the chloroform fraction being much better. These results show how important solvent polarity is for selectively extracting certain kinds of phytochemicals.

### 4.2.Comparison with Existing Studies

The results of this study are in line with and support the results of other recent studies on the phytochemistry and pharmacological importance of *Rauwolfia serpentina*. Notably, Jahan et al. (2022)[11] extracted flavonoids from the roots and validated their hypotensive effect through ACE inhibition, showing that the root extracts—especially in polar solvents—contain a wide range of bioactive components, such as alkaloids and flavonoids. The chloroform fraction in our analysis had a lot of alkaloids, which supports the idea that bioactivity depends on the solvent. Kamupini and Keshri (2024)[12] talked about how important it is to use different extraction methods and solvents with different polarities to improve therapeutic effectiveness. This is exactly what our results showed, with a statistically significant difference ($p = 0.001$) in alkaloid content across solvent systems. Khromov et al. (2025)[13] also wrote about alkaloid profiling and antiarrhythmic activity from *Rauwolfia serpentina* tissue cultures. They found compounds like reserpine and ajmaline, both of which were also successfully isolated and characterised in this study using advanced spectroscopic methods. Saini et al. (2024)[14] also looked at new developments in analytical methods for alkaloid analysis and stressed that NMR and MS are the best ways to get correct structural information. We used these methods successfully to confirm the identities of five important indole alkaloids. Lastly, Gadkari et al. (2024)[15] created and tested HPTLC methods for measuring yohimbine in *Rauwolfia serpentina* roots. This supports the idea that this compound exists and can be found, which we also found in our work. These investigations all corroborate the validity and usefulness of our methods and results, showing that they are in line with current research on the phytochemistry and pharmacognosy of *Rauwolfia serpentina*.

### 4.3.Implications of Findings

Finding important bioactive indole alkaloids in *Rauwolfia serpentina* has big effects on medication development and pharmaceutical research. Ajmaline and reserpine are two drugs that are known to lower blood pressure, prevent arrhythmias, and have neuropharmacological effects. This study backs up the plant's medicinal value and supports its continued use in herbal medicines and drug development pipelines. Also, the results show that extraction methods that use chloroform can increase the yield of target alkaloids, which is very important for getting the most out of extraction in both research and industrial settings. The method used here also shows that TLC-guided fraction monitoring and structure-based validation with integrated spectroscopic techniques can be used in future phytochemical research.

### 4.4.Limitations of the Study

Even though the results are promising, this study has certain problems. First, the study only looked at root samples from one place, which may not show the complete range of chemotypic differences in *Rauwolfia serpentina* across different areas. Second, only five alkaloids were found and identified. It's possible that certain alkaloids that were less active or worked better together were missed because of the way the study was set up or because they were in low amounts. Also, the study was mostly on identifying the structures of the compounds and didn't employ bioassays to test their pharmacological activity. This means that there is a gap in correlating chemical structure to biological function.

### 4.5.Suggestions for Future Research

Future research should include in vitro and in vivo pharmacological tests tos to see how the isolated alkURNs affect the body, especially when it comes to their ability to lower blood pressure and change the way the central nervous system works. Comparative studies that use samples from different geographic areas and ecological situations could assist determine chemotypic diversity and improve selection for usage in pharmaceuticals. Additionally, transcriptome and metabolomic methods could be used to look at the biosynthesis routes of alkaloids in *Rauwolfia serpentina*. Finally, researchers may look at green and scalable extraction methods like supercritical fluid extraction or microwave-assisted extraction to make natural product isolation more efficient and sustainable.

## 5. CONCLUSION

This work showed that it is possible to successfully extract, isolate, and determine the structure of the main bioactive alkaloids from the roots of *Rauwolfia serpentina*. Five alkaloids that are important to pharmacology—Reserpine, Ajmaline, Yohimbine, Serpentine, and Ajmalicine—were found using a systematic method that included solvent partitioning, column chromatography, and sophisticated spectroscopy analysis (FTIR, NMR, MS). Both the yield values and statistical validation showed that the chloroform fraction was the best solvent system for alkaloid enrichment. This work not only confirms that *Rauwolfia serpentina* has a lot of phytochemicals, but it also shows how modern phytochemical methods could be used to find new drugs from natural sources.

### 5.1.Summary of Key Findings

- A total methanolic crude extract of 312.5 g was obtained from *Rauwolfia serpentina* roots.
- Chloroform fraction yielded the highest alkaloid content (2.68%) among all tested solvents.
- Five distinct alkaloids were successfully isolated and structurally identified using TLC, FTIR, NMR, and MS.
- Statistical analysis (ANOVA) confirmed significant differences ($p = 0.001$) in alkaloid content among solvent fractions, validating chloroform as the optimal solvent.
- Spectral profiles matched known references, confirming the identity of isolated compounds with therapeutic relevance.

### 5.2.Significance of the Study

This work adds to the growing body of data that *Rauwolfia serpentina* is still a very important source of alkaloids that are important for medicine. The results not only support the plant's traditional medical applications, but they also show how phytochemical and spectroscopic approaches can be used to locate natural lead chemicals for modern medicines. The study also backs up the idea of choosing certain solvent systems based on the polarity of the compounds and gives future natural product investigations an approach that may be used again.

### 5.3.Recommendations

- Future studies should look at the biological activity of the isolated alkaloids (for example, cytotoxicity and antihypertensive assays) to find out how their structure affects their activity.
- To make sure that the quality of the raw materials is the same everywhere, the number of alkaloids should be checked in different places and at different times of the year.
- To make things more sustainable, we should use eco-friendly extraction processes like supercritical $CO_2$ or microwave-assisted extraction.
- Researchers may look at synergistic trials that combine isolated substances to see if they have stronger pharmacological effects.
- Working with the pharmaceutical industry could help produce standardised herbal formulations or semi-synthetic counterparts that can be used in clinical settings.

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