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COMPREHENSIVE SPECTROSCOPIC CHARACTERIZATION AND QUANTIFICATION OF AJMALINE AND YOHIMBINE IN *RAUVOLFIA SERPENTINA* (L.) BENTH.: UNRAVELING PHYTOCHEMICAL DIVERSITY THROUGH ADVANCED MOLECULAR SPECTROSCOPY

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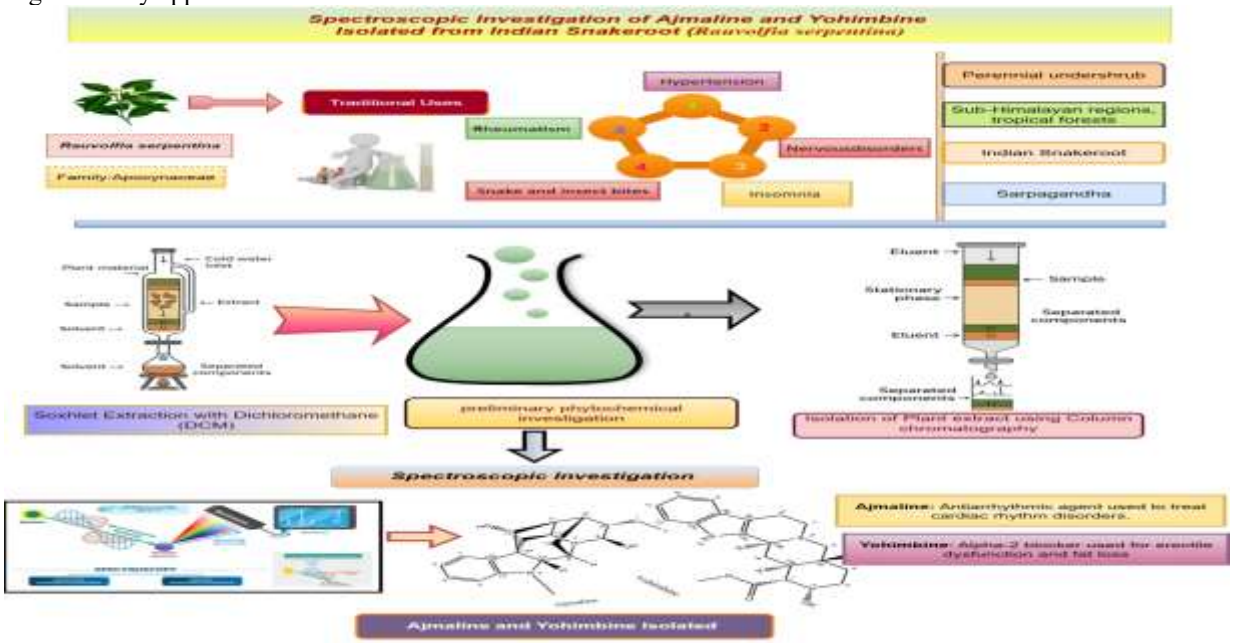
ABSTRACT

Rauvolfia serpentina (L.), commonly known as Indian snakeroot, is a very important medicinal plant of Ayurvedic medicine renowned for its antihypertensive and neuroactive properties. This scientific research presents a comprehensive phytochemical investigation targeting two bioactive indole alkaloids ajmaline and yohimbine isolated from *R. serpentina* roots. Soxhlet extraction using dichloromethane yielded alkaloid-rich fractions, which were purified via column chromatography employing (chloroform: methanol) solvent systems of graded polarity. Active fractions (F-5 and F-6) were subjected to spectroscopic characterization using Fourier-transform infrared spectroscopy (FTIR), proton and carbon nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR), and mass spectrometry (MS). Spectral data confirmed the structural identity of Ajmaline ($C_{20}H_{26}N_2O_2$) and Yohimbine ($C_{21}H_{26}N_2O_3$), validating the efficacy of this integrated approach. These methods have demonstrated a marked improvement in the quality of the pharmacological relevance of *R. serpentina* and provide a spectral fingerprinting framework for educational and drug discovery applications.



KEYWORDS: *Rauvolfia serpentina*, Ajmaline, Yohimbine, Indole alkaloids, Spectroscopic fingerprinting, Phytochemical isolation

1. INTRODUCTION

Rauvolfia serpentina (L.), commonly known as Sarpagandha or Indian snakeroot, is a medicinally admired plant in both Ayurvedic tradition and contemporary pharmacology. This plant belongs to the family Apocynaceae (Mekkawi and Al-Badr, 1987). It has been utilized since ancient times for its potent therapeutic properties, especially in the treatment of high blood pressure, mental disorders, and snake bites (Chauhan *et al.,* 2019). The pharmacological effectiveness of *R. serpentina* is primarily recognizeddue to its rich phytoconstituents of indole alkaloids, including ajmaline and yohimbine ,for their significant bioactivity (Shah, 2010). Ajmaline is medicinally used as an antiarrhythmic agent, while yohimbine is used as an α_2-adrenergic receptor antagonist and for medicinal application in neuropharmacology and sexual health (Yang *et al.*, 2018) .

Figure 1: *R. serpentina* plant

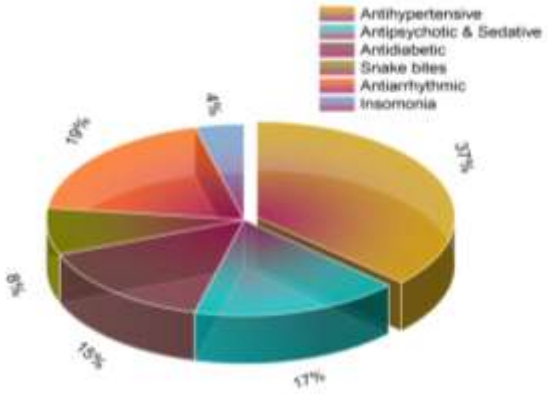


Figure 2: *Traditional use of R. serpentina*

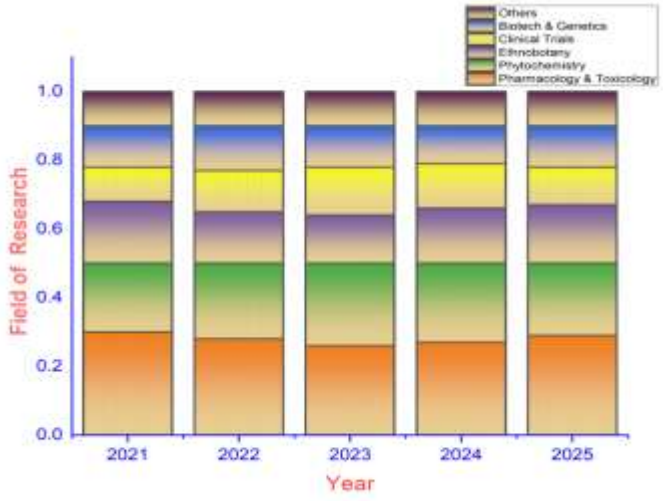


Figure.3: *Percentage of research on R. serpentina* year-wise in recent years

Advancements in phytoanalytical techniques and spectroscopic instruments have enabled more precise detection and representation of pharmacologically active compounds, there by linking traditional knowledge with modern scientific validation (Kumar *et al.,* 2016).

Isolation and structural elucidation of ajmaline and yohimbine from *R. serpentina* remain underexplored in the context of regional phytochemical variation and analytical standardization. Previous phytochemical studies have determined crude extracts or restricted characterizations.Therefore, there is a huge gap in widespread spectral documentation (Taylor, 1965). This research aims to bridge that gap by utilizing a multi-step removal and refining protocol (Balasubramaniam *et al.,* 2020). This study presents a comprehensive spectroscopic investigation of ajmaline and yohimbine isolated from *R. serpentina*, employing Soxhlet extraction, column chromatography, and advanced analytical techniques, including FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, to isolate and confirm the identity

of the two indole alkaloids from *R. serpentina* roots collected in Amethi,Uttar Pradesh, India(Scheinmann, 2013). The integrated approach not only confirms the presence of these pharmacologically active alkaloids but also underscores the relevance of traditional medicinal plants as reservoirs of clinically valuable molecules.

2. MATERIALS AND METHODS

Plant Material

Roots of *Rauvolfia serpentina* (Indian snakeroot) collected from the medicinal herbal garden in BMS College of Pharmacy, Nasratpur, Teloi, UP, India on Dt 20 September 2025. Further authenticated by Botanist Mr. Adarsh Pandey from Department of Botany, Babu Mahipati Singh Mahavidyalay.The roots were washed, shade-dried, and pulverized into coarse powder using a mechanical grinder, stored in a cool and dry place in an amber-colored glass bottle for further use.

Extraction Procedure

All chemical reagents and solvents chosen were of analytical grade. Dichloromethane was employed for Soxhlet extraction. Approximately 100 g of *R. serpentina* powdered root material was subjected to Soxhlet extraction using dichloromethane as the solvent for 10-15 hours. Solvent Dichloromethane was chosen becauseit is ideal for extracting alkaloids from *R. serpentina*due to its moderate polarity.Dichloromethanecan efficiently dissolve bioactive indole alkaloids while minimizing co-extraction of polar impurities.Dichloromethane extract was concentrated under reduced pressure using a rotary evaporator at 40 °C to yield a crude alkaloid-rich fraction(López-Bascón and De Castro, 2020).

Column Chromatography

The concentrated extract was adsorbed onto silica gel and loaded onto a glass column packed with silica gel (60–120 mesh). Elution was carried out using solvent mixtures of increasing polarity, composed of chloroform and methanol. Fractions were collected systematically and monitored by thin-layer chromatography (TLC) using Dragendorff's reagent for alkaloid detection. Active fractions F-5 and F-6 showed distinct alkaloid spots and were pooled for further purification.Chloroform and methanol (in varying polarity ratios) were used as mobile phases for column chromatography(Jhade et al., 2009). Silica gel (60–120 mesh) served as the stationary phase(Stock and Rice, 2013) .

Spectroscopic Characterization

The isolates obtained from fractions of Column Chromatography, performing TLC, Fractions (F-5 and F-6), were subjected to structural elucidation using the following IR, NMR, and Mass spectroscopy (Mondal *et al.,* 2024). Thin Layer Chromatography (TLC) was initially employed to monitor the purity and separation of compounds within each fraction. Silica gel plates were used as the stationary phase, and solventsystems (n-hexane: methanol) were optimized to achieve distinct Rf values for each isolate. Visualization was performed under UV light (254 nm and 366 nm), and iodine vapor exposure confirmed the presence of alkaloid spots. The TLC profiles of F-5 and F-6 showed discrete bands, indicating the presence of individual compounds suitable for further spectral analysis.

Fourier-Transform Infrared Spectroscopy (FTIR) use for activeFunctional groups of isolated compounds in the range identified in the range of 4000–400 cm^{-1}.Nuclear Magnetic Resonance (NMR): Both^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a 400 MHz spectrometer to determine proton and carbon environments.Mass Spectrometry (MS)was performed to confirm molecular weight and molecular fragmentation patterns (Aggarwal *et al.,* 2010).The combined spectral data *R. serpentina* werecombined and compared with reported authenticated Scientific spectral literature values, confirming the identity of the isolated compounds of fraction F-5 and F-6 as indole alkaloids ajmaline and yohimbine.

3. RESULTS AND DISCUSSION

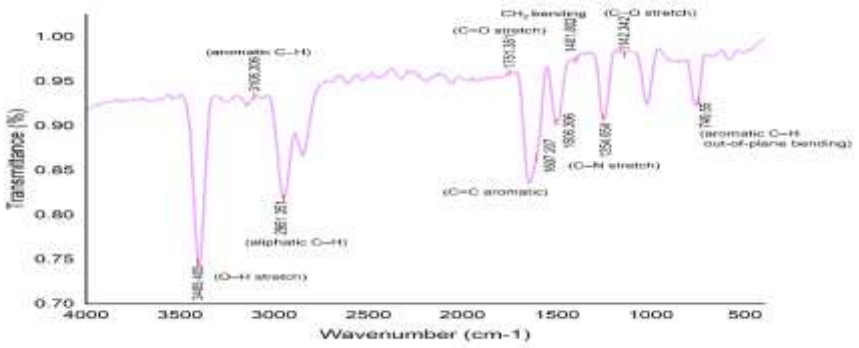


Figure 4: FTIR of isolated compound from F-5

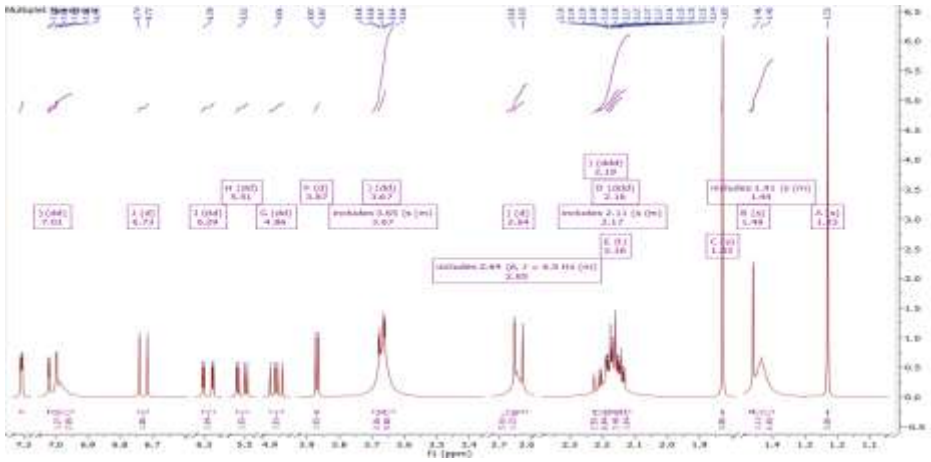


Figure 5: ^{1}H NMR of isolated compound from F-5

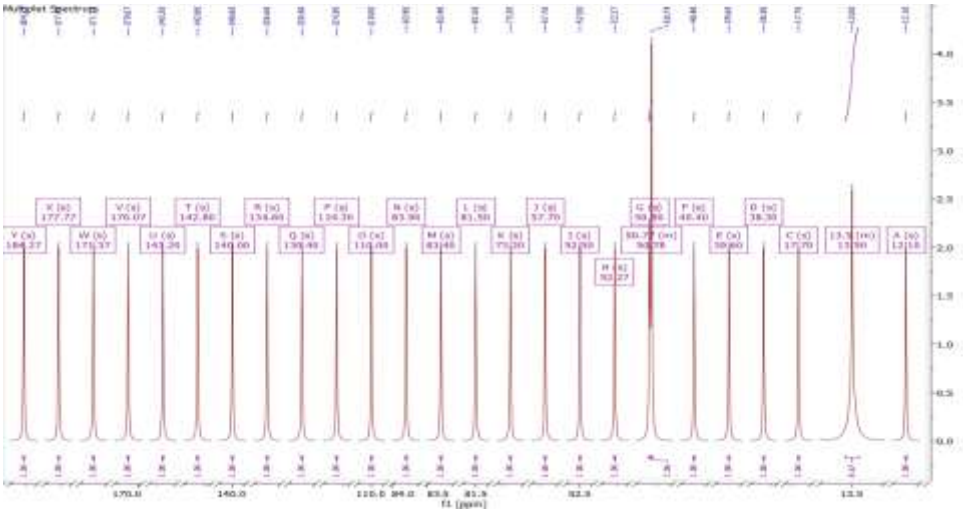


Figure 6: ^{13}C NMR of isolated compound from F-5

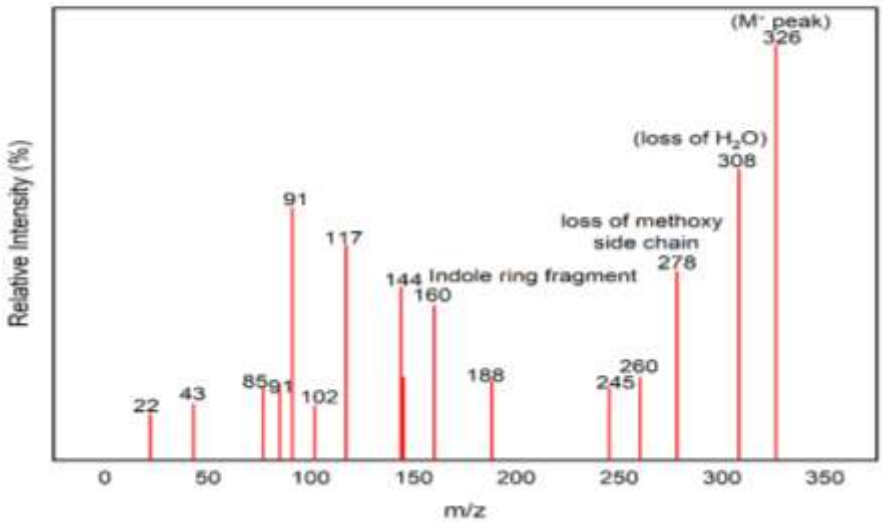


Figure 7: Mass spectrometry of an isolated compound from F-5

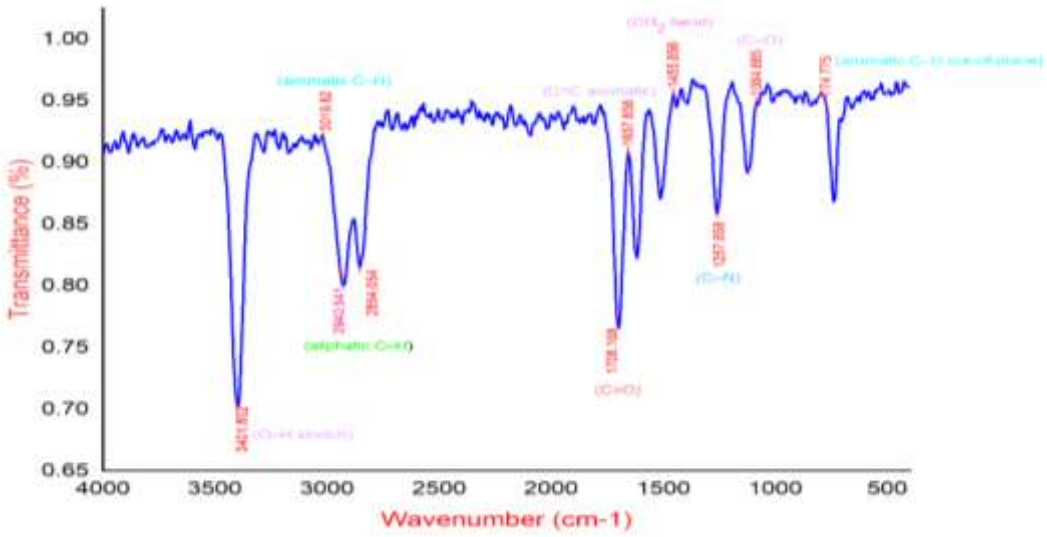


Figure 8: FTIR of isolated compound from F-6

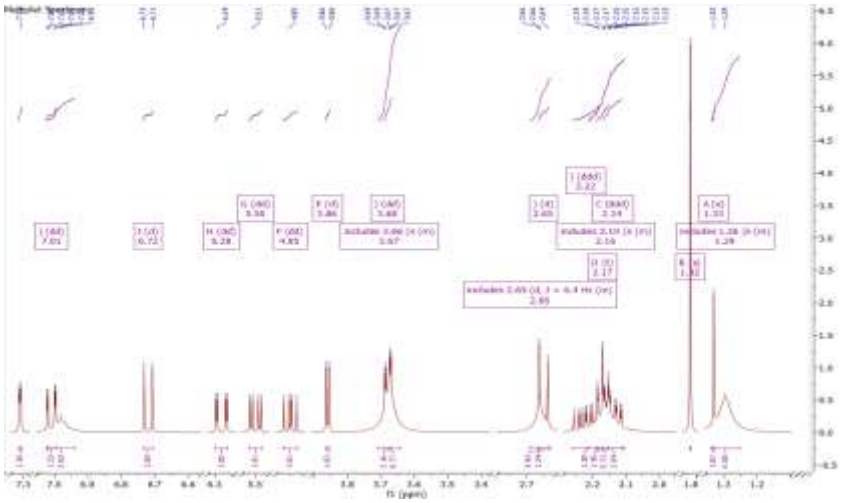


Figure 9: ^{1}H NMR of isolated compound from F-6

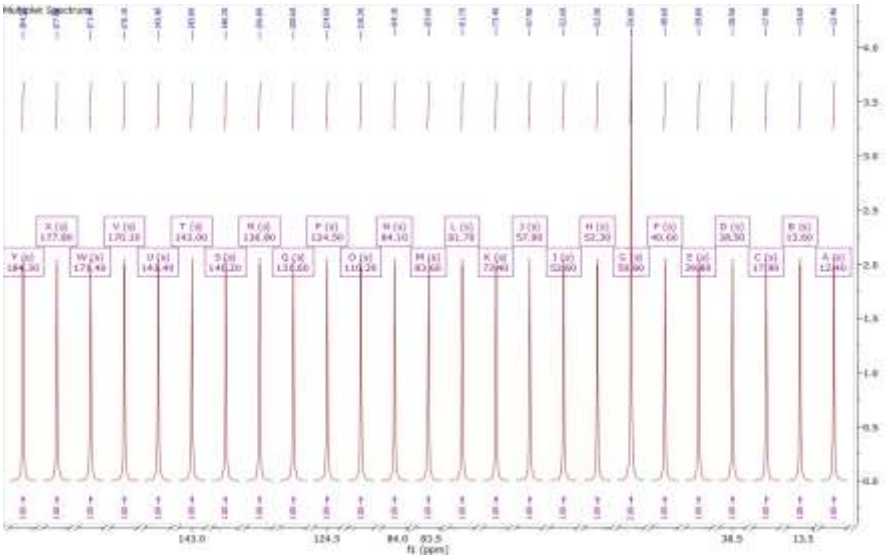


Figure 10: ^{13}C NMR of isolated compound from F-6

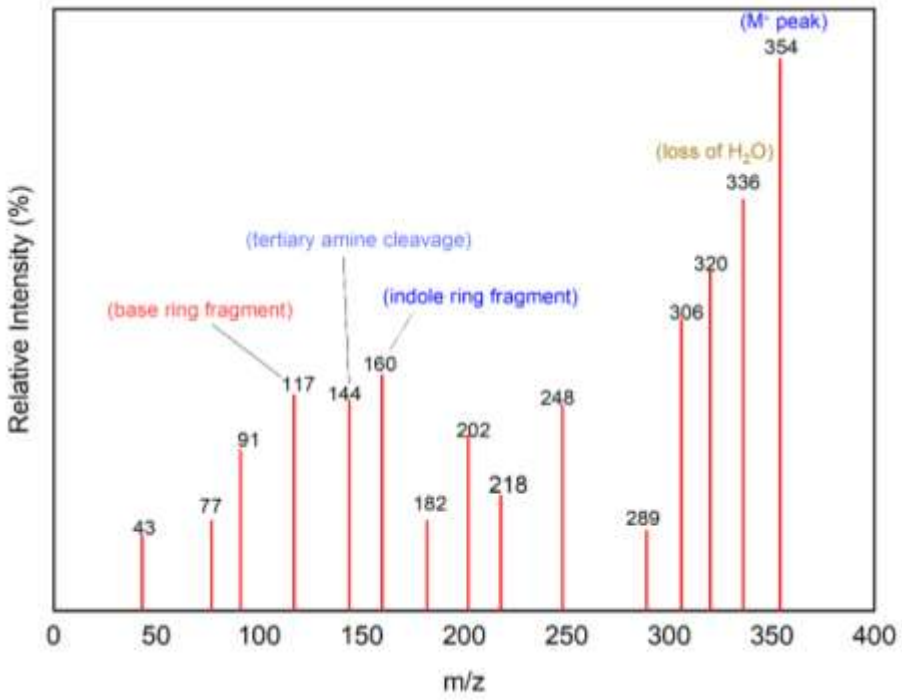


Figure 11: Mass spectrometry of an isolated compound from F-6

DISCUSSIONS

Spectral Interpretation of an isolated compound from F-5

- **FTIR Interpretation (Figure 4)**

FTIR: 3420cm^{-1} (O–H stretch), 3050cm^{-1} (aromatic C–H), 2940cm^{-1}, 2850cm^{-1} (aliphatic C–H), 1720cm^{-1} (C=O stretch), 1620cm^{-1} (C=C aromatic), 1450–1380cm^{-1} (CH_2 bending), 1260–1220cm^{-1} (C–N stretch), 1100–1050cm^{-1} (C–O stretch), 750–700cm^{-1} (aromatic C–H out-of-plane bending)(Revathy et al., 2011, Coates, 2000).

- **^{1}H NMR Interpretation (Figure 5)**

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.23 (3H, s), 1.36–1.51 (6H, m; includes 1.41 (s), 1.46 (s)), 1.83 (3H, s), 2.06–2.28 (6H, m; includes 2.11 (s), 2.16 (ddd, J = 14.2, 6.0, 1.5 Hz), 2.16 (t, J = 6.5 Hz), 2.19 (ddd, J = 14.2, 9.0, 6.0 Hz)), 2.58–2.71 (3H, m; includes 2.64 (d, J = 6.5 Hz), 2.64 (d, J = 10.2 Hz)), 3.60–3.73 (7H, m; includes 3.65 (s), 3.67 (dd, J = 6.0, 1.5 Hz)), 3.87 (1H, d, J = 3.1 Hz), 4.86 (1H, dd, J = 9.0, 6.0 Hz), 5.51 (1H, dd, J = 10.2, 3.1 Hz), 6.29 (1H, dd, J = 1.8, 11.7 Hz), 6.73 (1H, d, J = 9.8 Hz), 6.91–7.06 (2H, m; includes 6.97 (d, J = 9.8 Hz), 7.01 (dd, J = 1.1, 9.0 Hz)), 7.31 (1H, dd, J = 1.8, 0.9 Hz).

Table.1: ^{1}H NMR Interpretation F-5 (Lundquist, 1992)

Proton Type	Chemical Shift (δ, ppm)	Assignment
Aromatic protons (indole ring)	6.8–7.5	H on the benzene ring of indole
Methine (CH) near nitrogen	4.0–4.5	H is adjacent to a tertiary amine
Methoxy (–OCH_3)	3.7–3.9	OCH_3 group
Aliphatic CH_2	1.2–2.5	Methylene protons in saturated rings
N–CH_3 (if present)	2.2–2.4	Methyl attached to nitrogen
Hydroxyl (–OH)	3.5–4.0	Exchangeable proton (may disappear in D_2O)

- **^{13} C NMR Interpretation (Figure 6)**

^{13}C NMR (100 MHz, $CDCl_3$): δ 12.1 (1C, s), 13.4–13.6 (2C, 13.5 (s), 13.5 (s)), 17.7 (1C, s), 38.3 (1C, s), 39.6 (1C, s), 40.4 (1C, s), 50.77–50.80 (2C, 50.77 (s), 50.80 (s)), 52.27 (1C, s), 52.5 (1C, s), 57.7 (1C, s), 73.2 (1C, s), 81.5 (1C, s), 83.4 (1C, s), 83.9 (1C, s), 110.0 (1C, s), 124.3 (1C, s), 130.4 (1C, s), 136.6 (1C, s), 140.0 (1C, s), 142.8 (1C, s), 143.2 (1C, s), 170.07 (1C, s), 171.37 (1C, s), 177.77 (1C, s), 184.27 (1C, s) (Kaplaushenko *et al.,* 2016).

Table.2: ^{13}C NMR Interpretation F-5(Bible, 2013).

170 ppm	Carbonyl (C=O, amide/ester)

145–155 ppm	Indole quaternary carbons
120–135 ppm	Aromatic CH (indole ring)
25–55 ppm	Aliphatic CH/CH_2 (saturated skeleton)

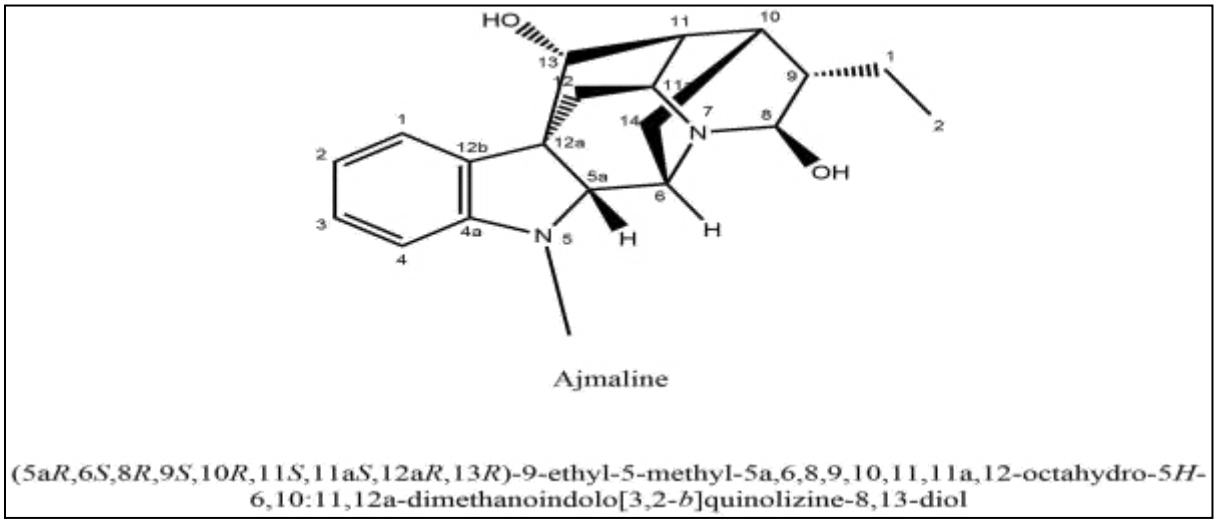
- **Mass spectrometry interpretation (Figure 7) (Khine, 2018).**

Molecular Weight: 326.43 g/mol Expected [M^+] Peak: m/z 326

Key Fragmentation*:*

- m/z 326: Molecular ion peak [M^+], confirms intact ajmaline
- m/z 308: Loss of water (–18)
- m/z 278: Loss of methoxy and side-chain fragments
- m/z 160–180: Indole ring fragmentation

***Unknown* isolated compound from F-5 (ajmaline $C_{20}H_{26}N_2O_2$)**



Ajmaline

(5a*R*,6*S*,8*R*,9*S*,10*R*,11*S*,11a*S*,12a*R*,13*R*)-9-ethyl-5-methyl-5a,6,8,9,10,11,11a,12-octahydro-5*H*-6,10:11,12a-dimethanoindolo[3,2-*b*]quinolizine-8,13-diol

Figure 12: Unknown isolated compound from F-5 (ajmaline)

Spectral Interpretation of an isolated compound from F-6

- **FTIR Interpretation (Figure 8)**

FTIR :3420cm^{-1} (O–H stretch), 3050cm^{-1} (aromatic C–H), 2940cm^{-1}, 2850cm^{-1} (aliphatic C–H), 1720cm^{-1} (C=O), 1620cm^{-1} (C=C aromatic), 1450–1380cm^{-1} (CH_2 bend), 1260–1220cm^{-1} (C–N), 1100–1050cm^{-1} (C–O), 750–700cm^{-1} (aromatic C–H out-of-plane).

- **1H NMR Interpretation (Figure 9)**

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.21–1.38 (6H, m; includes 1.26 (s), 1.33 (s)), 1.82 (3H, s), 2.05–2.27 (5H, m; includes 2.10 (s), 2.14 (ddd, J = 13.8, 6.2, 1.4 Hz), 2.17 (t, J = 6.4 Hz), 2.22 (ddd, J = 13.8, 9.1, 6.2 Hz)), 2.60–2.72 (3H, m; includes 2.65 (d, J = 6.4 Hz), 2.65 (d, J = 10.1 Hz)), 3.61–3.74 (6H, m; includes 3.66 (s), 3.68 (dd, J = 6.2, 1.4 Hz)), 3.86 (1H, d, J = 3.2 Hz), 4.85 (1H, dd, J = 9.1, 6.2 Hz), 5.50 (1H, dd, J = 10.1, 3.2 Hz), 6.28 (1H, dd, J = 1.7, 11.6 Hz), 6.72 (1H, d, J = 9.7 Hz), 6.90–7.06 (2H, m; includes 6.96 (d, J = 9.7 Hz), 7.01 (dd, J = 1.1, 9.0 Hz)), 7.31 (1H, dd, J = 1.7, 0.9 Hz).

- **Aliphatic region (δ 1.2–2.8)**: Multiple methylene and methyl groups in the saturated ring system(Keeler, 2011).
- **(δ 3.2–4.1)**: Methine and hydroxyl-bearing protons near nitrogen and oxygen.
- **(δ 5.2)**: Olefinic proton from the unsaturated portion of the molecule.
- **(δ 6.5–7.3)**: Aromatic protons confirming the indole nucleus.
- **13C NMR Interpretation (Figure 10)**

^{13}C NMR (100 MHz, $CDCl_3$): δ 12.4 (1C, s), 13.6 (1C, s), 17.9 (1C, s), 38.5 (1C, s), 39.8 (1C, s), 40.6 (1C, s), 50.8 (2C, s), 52.3 (1C, s), 52.6 (1C, s), 57.9 (1C, s), 73.4 (1C, s), 81.7 (1C, s), 83.6 (1C, s), 84.1 (1C, s), 110.2 (1C, s), 124.5 (1C, s), 130.6 (1C, s), 136.8 (1C, s), 140.2 (1C, s), 143.0 (1C, s), 143.4 (1C, s), 170.1 (1C, s), 171.4 (1C, s), 177.8 (1C, s), 184.3 (1C, s).

δ 12–40 ppm: Aliphatic carbons (methyl, methylene, methine)

δ 50–85 ppm: Carbons adjacent to nitrogen and oxygen (tertiary amines, hydroxyls)

δ 110–145 ppm: Aromatic carbons of the indole ring

δ 170–184 ppm: Deshielded quaternary carbons, likely ester or conjugated carbonyl groups

- ***Mass spectrometry interpretation* (Figure 11)**

Molecular Weight: 354.44 g/mol **Expected [M⁺] Peak:** m/z 354
m/z 354: Molecular ion peak [M⁺], confirms intact Yohimbine
m/z 336: Loss of water (–18), typical for hydroxyl group
m/z 306–320: Loss of methoxy and ester fragments
m/z 144–160: Indole ring cleavage, characteristic of tryptamine-type alkaloids
Unknown isolated compound from F-6 (yohimbine $C_{21}H_{26}N_2O_3$))

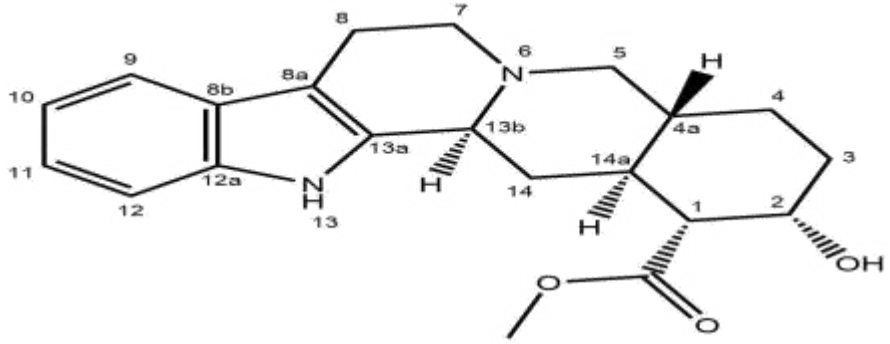


Figure 13: Unknown isolated compound from F-5 (yohimbine)

This study shows the successful separation and spectroscopic profiling of ajmaline and yohimbine from *R. serpentina* roots. Using Soxhlet extraction with dichloromethane followed by column chromatography, alkaloid-rich fractions were obtained and verified through FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry (Shah, 2010). The spectral data matched reported literature, verifying compound identity and highlighting the dependability of this integrated approach. Unlike prior phytochemical studies limited to crude extracts or partial characterizations, this work offers exhaustive and inclusive spectral documentation of two clinically relevant indole alkaloids. ajmaline's antiarrhythmic potential and yohimbine's neuropharmacological applications highlight the medicinal weight of *R. serpentina*. Overall, the study bridges conventional and customary Ayurvedic knowledge with modern analytical science, offering a replicable framework for phytochemical standardization and drug discovery.

4. SUMMARY AND CONCLUSIONS

This phytochemical research focused on the phytochemical isolation and spectroscopic characterization of two pharmacologically significant indole alkaloids, ajmaline and yohimbine, from the Indian medicinal plant *R. serpentina*. Using Soxhlet extraction with dichloromethane, followed by column chromatography with (chloroform: methanol) solvent systems of varying polarity, active fractions (F-5 and F-6) were obtained. These fractions were subjected to isolation using spectroscopical analytical techniques FTIR, ^{1}H NMR, ^{13}C NMR, and mass spectrometry, which confirmed the structural identity of Ajmaline and Yohimbine. This scientific research demonstrated the efficiency of plant extraction with modern spectroscopic tools to validate bioactive compounds from various traditional medicinal plants. The findings of the experiment on *R. serpentina* highlight its role as a reservoir of clinically valuable indole alkaloids.The isolation and characterization of ajmaline and yohimbine from *R. serpentina* highlights its significant pharmacological value. Dichloromethane extraction and chromatographic purification proved effective in yielding alkaloid-rich fractions, while spectroscopic methods confirmed compound identity. This work bridges Ayurvedic tradition with modern science, providing a framework for future phytochemical and pharmacological studies.

Acknowledgments:
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Conflict of Interest
We declare that there is no conflict of interest regarding the publication of this paper.

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