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Research Article

Isolation and spectroscopic characterization of indole alkaloids and coumarin from *Rauvolfia verticillata* (Lour.) Baill.

Nisha Ayyappan[1], Ramasubbu Raju[2], E. G. Wesely Jebasing Devairakkam[1]*

[1]*Department of Botany, Arignar Anna Government Arts College, Namakkal-637002, Tamil Nadu, India*
[2]*Department of Biology, The Gandhigram Rural Institute (Deemed to be University), Dindigul-624302, Tamil Nadu, India*

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*Corresponding authors: E. G. Wesely Jebasing Devairakkam, E-mail: egwesely1@gmail.com

Abstract

Rauvolfia verticillata is a highly significant and rare medicinal shrub belonging to the family Apocynaceae, renowned as a primary source of pharmacologically active indole alkaloids. This study aims to isolate and characterize the bioactive secondary metabolites derived from the methanolic stem extract of *R. verticillata.* To achieve this, the dried stem powder was subjected to exhaustive extraction with methanol. The crude methanolic extract was subsequently fractionated via column chromatography. The resulting fractions were monitored and evaluated using Thin-Layer Chromatography (TLC); fractions exhibiting a single, distinct band were identified as isolated, homogenous compounds. Through this chromatographic workflow, two major indole alkaloids, reserpine and yohimbine and one coumarin derivative, seselin, were successfully isolated. The structural elucidation and chemical integrity of these pure compounds were comprehensively confirmed using an array of spectroscopic and spectrometric techniques, including Ultraviolet-Visible (UV-Vis) spectroscopy, Liquid Chromatography-Mass Spectrometry (LC-MS), Fourier-Transform Infrared (FT-IR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy. To fully ascertain the therapeutic potential of the extract and its isolated compounds, future investigations will focus on evaluating their toxicity profiles and conducting in vivo pharmacological studies.

Keywords: TLC, Methanolic extract, Indole alkaloids, Coumarin, Reserpine, Yohimbine, Seselin

Introduction

Rauvolfia verticillata (Lour.) Baill. is a traditional Chinese medicinal herb and a significant source of heterocyclic indole alkaloids (Gao *et al.,* 2015). It is a shrub species in the family Apocynaceae and is found throughout the Indo-China peninsula, Indonesia, the Philippines, Thailand, Vietnam, India, and Sri Lanka. It is commonly known as common devil pepper. The flowers are white, with ovate lobes on the corolla and 1 cm long, glabrous, reddish pedicles. Inflorescences may yield at least 35 flowers. The fruits are oblong, 10×7 mm and black. The species is used therapeutically in China to treat typhus, malaria, and snakebite (Chen *et al.,* 2019). The extract was first used in China in the 1950s to effectively treat several skin conditions characterised by redness and itching, with minimal side effects (Hong *et al.,* 2016). The species of the family Apocynaceae are used in traditional medicine to treat gastrointestinal problems (Rohela *et al.,* 2016). The German physician and explorer Leonhard Rauwolf, who wrote a book detailing his extensive travels in 1582, is the source of the genus name *Rauwolfia*, which was later changed to *Rauvolfia* (Kumari *et al.,* 2021; Saranya *et al.,* 2021; Ayyappan *et al.,* 2025).

Members of the genus *Rauvolfia* are well known for their abundance of specialised heterocyclic alkaloids with a monoterpene-indole skeleton (Gao *et al.,* 2011). Rauvolfia extract has been used as a medicine for hypertension (Hong *et al.,* 2010). Reserpine was one of the first oral drugs for hypertension that worked well and made a significant contribution to early psychiatric therapy (Katzung, 2021). The most well-known alkaloid reserpine helps to treat mental problems, cardiovascular ailments and hypertension. It is also a tranquillizer that is highly valued by the current pharmaceutical sector (Sulaiman *et al.,* 2020). Due to its sedative and antihypertensive properties, reserpine is a highly effective therapeutic drug (Dey & Pandey, 2014). Yohimbine is known for its pharmacological activity on the central and peripheral nervous systems. It acts as a selective α2-adrenergic receptor antagonist, increasing sympathetic outflow and norepinephrine release (Katzung, 2021). It has also been studied for its efficacy in treating orthostatic hypotension and some types of sexual dysfunction (Tam *et al.,* 2001; Saranya *et al.,* 2021; Ayyappan *et al.,* 2025). Coumarins are a significant group of plant-derived secondary metabolites known for their various pharmacological activity and therapeutic potential. Seselin has been evaluated for its cytotoxicity against certain cancer cell lines, demonstrating its potential as a lead compound in drug development (Venugopala *et al.,* 2013). It also has significant biological activities, including antioxidant, antimicrobial, anti-inflammatory, and potential anticancer effects (Mousavi *et al.,* 2015). In the present study, two bioactive indole alkaloids and one coumarin compound were successfully isolated and characterised from the methanolic stem extract of *R. verticillata.* The structures of the isolated compounds were confirmed by spectroscopic analysis (UV-Vis, FT-IR, LC-MS, and NMR).

Materials and methods

Plant materials collection and extraction

The stem of *Rauvolfia verticillata* was collected in the Megamalai Hills, Theni district, Tamil Nadu, India,

and identified and authenticated at the Gandhigram Rural Institute (Deemed to be University), Tamil Nadu, India. The voucher specimens of *R. verticillata* were stored in the herbarium (GUD) of the Department of Biology. The collected stems were shade-dried, pulverised into a fine powder, and stored in an airtight container. Methanol was chosen as the solvent for extraction due to its high polarity, strong penetration, and ability to extract a wide range of bioactive secondary metabolites. The finely powdered sample was extracted with methanol for further progress. The powdered material was immersed in methanol for 72 hours, then filtered through a Whatman No. 1 filter paper over 3 days. The process was repeated 3 to 4 times, and the extracts were concentrated at 45 °C under reduced pressure using a vacuum rotary evaporator (Buchi RotavaporR-300). The concentrated extract was refrigerated at 4 °C for further analysis.

Isolation and purification of bioactive compounds

The methanolic stem extract was fractioned by silica gel column chromatography (150 g, 60-120 mesh) using gradient elution. The column was packed with hexane, and vigorous swirling was performed to create a silica gel slurry, which was then poured into the column. An equal amount of semisolid extract and silica gel was mixed and the extract with silica mixture (2 g) was gently put into the column. Fractions were eluted using solvent systems of increasing polarity (hexane, ethyl acetate, chloroform, acetone, and methanol) with gradient mixtures of hexane: ethyl acetate, ethyl acetate: chloroform, chloroform: acetone, and acetone: methanol (40:0-0:40). Forty fractions (40 mL each) were collected per system and analysed individually by Thin Layer Chromatography (TLC). The collected fractions were spotted on TLC sheets and run through several mobile-phase solvent systems. The fractions with similar Rf values were combined and treated as a single compound. The compounds (1 mg/mL in DMSO) were analyzed by UV–Vis spectroscopy (200-800 nm; GENESYS 180) using the solvent as blank. FT-IR spectra (450-5000 cm^{-1}) were recorded on a Jasco FT/IR-4700. NMR spectra were obtained in $CDCl_3$ on a Bruker Avance 400 MHz instrument using TMS as internal standard (δ, ppm). LC-MS analysis (Agilent 6530 LC/Q-TOF) was performed with methanolic samples (10 mg) at a flow rate of 0.1-1 mL/min. All structural characterization studies were carried out at The Gandhigram Rural Institute (Deemed to be University), Dindigul, Tamil Nadu, India.

Reserpine: White to pale yellow powder; melting point: 262-266 °C; UV(MeOH) λ_{max}:230,228 nm; IR (KBr) ν_{max}: 3822,3430,2840,1710,1269,659 cm^{-1}; ESIMS (pos.): m/z 609.2$[M+H]^+$;^{1}H NMR: Table 1 and ^{13}C NMR: Table 2 (calculated for $C_{33}H_{40}N_2O_9$).

Yohimbine: White to off-white crystalline powder; melting point: 231-233 °C; UV(MeOH) λ_{max}: 232,234 nm; IR (KBr) ν_{max}: 3158,1715,1294,1199,1054,696 cm^{-1}; ESIMS (pos.): m/z 355$[M+H]^{+;1}$H NMR: Table 3 and ^{13}C NMR: Table 4 (calculated for $C_{21}H_{26}N_2O_3$).

Table 1: ^{1}H-NMR for Reserpine

Chemical Shift (ppm)	Integration	Assignment
7.0-7.5	4H (m)	Aromatic protons from the indole-fused benzoring system
7.263	1H (s)	$CDCl_3$ solvent peak
3.51-3.92	6H (s)	Six methoxy protons (-OCH_3) from different groups
1.5-3.2	_ (m)	Overlapping methylene and methine protons (yohimbane core)
7.32-7.33	2H (s)	Symmetric aromatic protons (3,4,5-trimethoxybenzoate)

Table 2: ^{13}C-NMR for Reserpine

Chemical shift (ppm)	Assignment	Significance
>165 ppm	Carbonyl carbons (methyl ester & trimethoxybenzoate)	Key ester carbonyl resonances confirming the lactone structure
110-155 ppm	Aromatic/olefinic carbons	Defines the aromatic and olefinic framework of coumarin and pyran ring.
77.02	$CDCl_3$ solvent triplet	Standard solvent peak used as a reference
56.28	Methoxy carbons (-OCH_3)	Aliphatic methoxy groups integrated in the core structure
20-60	Methylene/methine carbons (CH_2/CH)	Complex framework of fused yohimbane ring system

Table 3: ^{1}H-NMR for Yohimbine

Chemical Shift (ppm)	Integration	Assignment
7.0-7.5	4H (Complex)	Aromatic protons of fused benzoring (indole core)
7.26	1H (s)	$CDCl_3$ solvent peak
5.71-7.61	_ (m)	Coumarin lactone double bond and fused benzene/pyran ring
6.23	_ (d)	Cis-coupled protons of the pyrone ring (H-3)
7.60	_ (d)	Cis-coupled protons of the pyrone ring (H-4)
5.73	_ (d)	Olefinic protons of the fused dimethylpyran ring
6.89	_ (d)	Olefinic protons of the fused dimethylpyran ring
3.7-3.8	_ (s)	Carbomethoxy methyl group (-COOCH3)
4.2	_ (s)	Oxygenated methine proton (H-17) near hydroxyl
1.48	6H (s)	Gem-dimethyl groups (unique to the pyran ring)

Seselin: White colour powder; melting point: 119-124 °C; UV(MeOH) λ_{max}: 292, 288 nm; IR (KBr) ν_{max}: 3773, 3423, 2452, 699, 649 cm^{-1}; ESIMS (pos.):*m/z* 251 $[M+H]^+$; ^{1}H NMR: Table 5 and ^{13}C NMR: Table 6 (calculated for $C_{14}H_{12}O_3$).

Results

Using column chromatography and thin-layer chromatography (TLC), three compounds were isolated

Table 4: ^{13}C-NMR for Yohimbine

Chemical shift (ppm)	Assignment	Significance
175.8	Ester carbonyl carbon (-$COOCH_3$)	Confirms the carbomethoxy group at the lactone position
110-140	Aromatic/olefinic carbons	Defines the indole core and fused pyran ring
77.02	$CDCl_3$ solvent triplet	Standard reference peak for solvent signal
21-67	Aliphatic carbons (CH_2/CH)	Represents the complex methylene/methine framework
52	Methoxy methyl carbon (-OCH_3)	Confirms ester-bound methoxy group at C-17

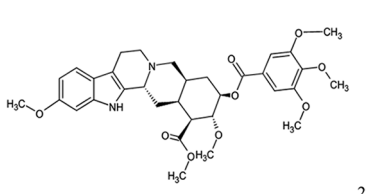
Table 5: ^{1}H-NMR for Seselin

Chemical Shift (ppm)	Integration	Assignment
5.71-7.61	_ (m)	Aromatic and olefinic protons (coumarin lactone and fused ring)
7.26	1H (s)	$CDCl_3$ solvent peak
6.23	_ (d)	Cis-coupled protons of the pyrone ring (H-3)
7.60	_ (d)	Cis-coupled protons of the pyrone ring (H-4)
5.73	_(d)	Olefinic protons of the dimethylpyran ring
6.89	_(d)	Olefinic protons of the dimethylpyran ring
1.48	6H (s)	Gem-dimethyl groups (two-equivalent) in the pyran ring

Table 6: ^{13}C-NMR for Seselin

Chemical shift (ppm)	Assignment	Significance
171.88	Lactone carbonyl carbon (C-2)	Confirms the central pyrone ring lactone carbon
100-160	Aromatic/ olefinic carbons	Defines the coumarin skeleton and fused dimethylpyran ring
109.80	Aromatic methine carbon	Electron-rich aromatic carbon in the ring
77.02	$CDCl_3$ solvent triplet	Standard reference peak for solvent signal
20-30	Gem-dimethyl carbons (C-2')	Confirms gem-dimethyl groups attached to pyran carbon (C-2')

from the methanolic stem extract of *R. verticillata*. The compounds were confirmed by spectroscopic analysis, namely UV-Vis, FT-IR, LC-MS and ^{1}H and ^{13}C NMR.



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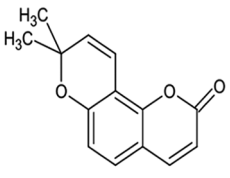

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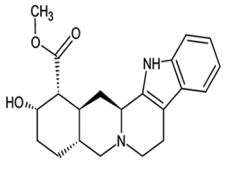

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Compound 1: Reserpine

The dark brown fraction was obtained from the liquid-liquid fraction using a mixture of methanol and chloroform. TLC analysis of 40 fractions yielded 15-20 bands. Fractions with similar Rf values were pooled and purified by repeated column chromatography, yielding 5 fractions by TLC. Fraction F1, showing fewer bands, was subjected to confirmatory analyses and identified as an indole alkaloid, reserpine.

The UV–Vis spectroscopy showed a λmax at 230 nm, indicating π–π* transitions and the presence of aromatic or conjugated systems (Figure 1a). The FTIR spectrum of reserpine exhibited peaks at 3822.22 and 3430.74, ascribed to the stretching vibration of the N-H amine group. The peaks at 2937.05 and 2840.63 showed C-H stretching. The stretching vibration of the C=O group was observed at 1710.55 cm^{-1}. The peak at 1621.84 showed a stretching of the C=C group. The C-H bending was observed at the peaks of 1453.1 and 1411.64 cm^{-1}. The peaks at 1269.9 and 1221.68 correspond to C-N stretching vibrations. The peaks at 1062.58 and 1029.8 corresponded to C-O stretching. The peaks at 760.78, 659.53, and 617 correspond to C-H bending vibrations (Figure 2a). LC-MS spectroscopy was performed in positive ionisation mode. The peak observed at 609.2 corresponds to the molecular ion peak (M^+). The peaks were recorded at 116.10, 242.2, 355.20 and 1253.53, corresponding to fragmentation peaks (Figure 3a).

Based on the provided Bruker 400 MHz ^{1}H-NMR spectrum recorded in CDCl3, the plot reveals the highly complex structural framework of Reserpine, a prominent indole alkaloid. The spectrum exhibits downfield aromatic proton signals between δ 6.5 ppm and 7.5 ppm, including a distinct pair of symmetric aromatic protons from the 3,4,5-trimethoxybenzoate ring appearing as a singlet near δ 7.32-7.33 ppm, alongside the standard residual solvent peak of $CDCl_3$ at δ 7.263 ppm. A highly diagnostic feature of this spectrum is the intense cluster of methoxy singlets clustered between δ3.51 ppm and 3.92 ppm, representing the six separate methoxy groups (-OCH_3) integral to reserpine's architecture: four belonging to the trimethoxybenzoate moiety, one attached to the indole ring, and one from the methyl ester functional group. Upfield of these peaks, the highly complex, overlapping multiplets spanning the aliphatic region from δ 1.5 ppm to 3.2 ppm map out the dense arrangement of methylene and methine protons that constitute the rigid, fused yohimbane core system (Figure 4).

^{13}C-NMR spectra recorded using a power-gated proton-decoupling sequence (zgpg30) provide definitive evidence for the highly oxygenated carbon architecture of reserpine. The spectrum exhibits key downfield carbonyl resonances above δ165 ppm (typically mapping to the two distinct ester carbonyl carbons: the methyl ester and the 3,4,5-trimethoxybenzoate linkage), while the complex array of peaks spanning the δ 110-155 ppm region maps out the

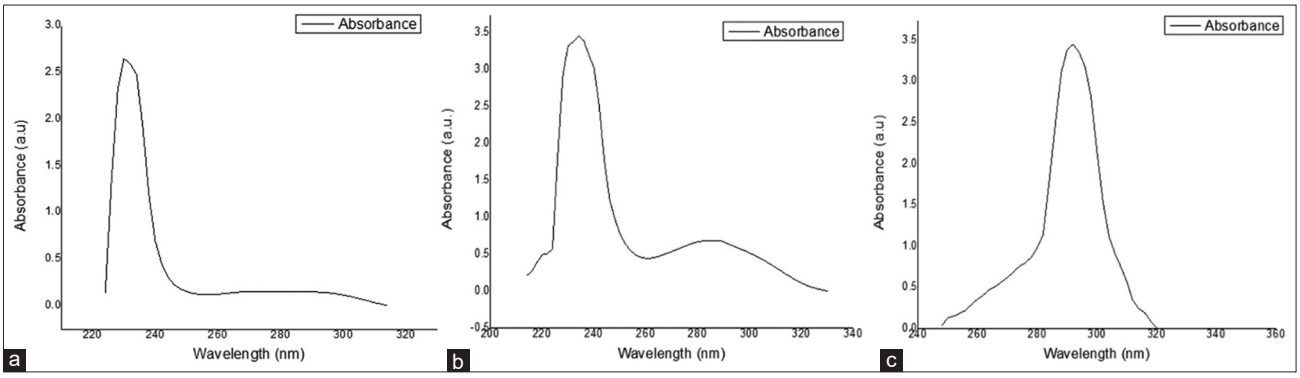

Figure 1: UV-Visible spectrum of a) Reserpine, b) Yohimbine and c) Seselin

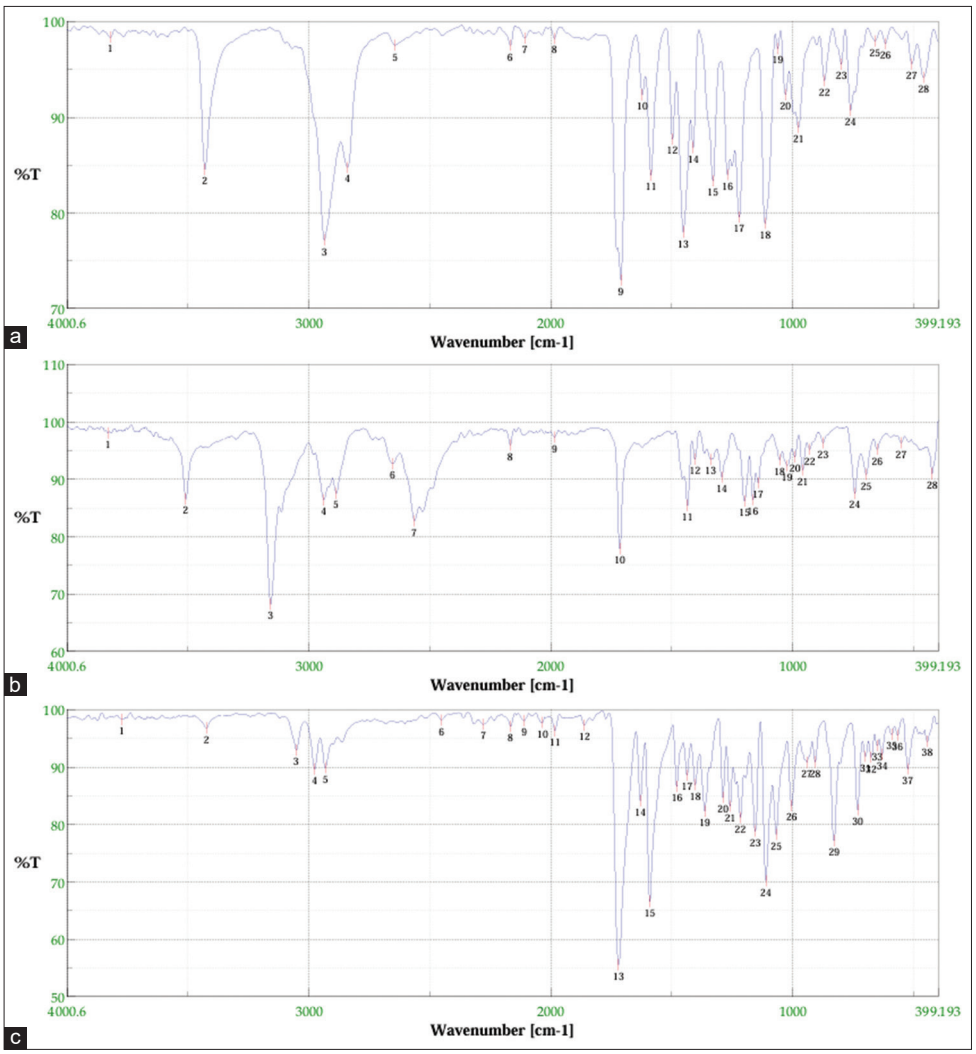

Figure 2: FTIR spectrum of a) Reserpine, b) Yohimbine and c) Seselin

aromatic and olefinic carbons belonging to both the indole system and the tri-substituted trimethoxybenzoate phenyl ring. The characteristic triplet centred at δ 77.02 ppm confirms the presence of the $CDCl_3$ NMR solvent. Most diagnostically, a sharp, highly intense signal cluster resolves prominently near δ 56.28 ppm, which matches the multiple equivalent aliphatic methoxy carbons ($-OCH_3$) distributed across reserpine's core, appearing alongside the broader upfield multiplex (δ 20-60 ppm) that represents the intricate system of methylene (CH_2) and methine (CH) carbons making up its fused yohimbane ring skeleton (Figure 4).

Compound 2: Yohimbine

A dark brown fraction was obtained by column chromatography using acetone and chloroform to separate the liquid-liquid fraction from *R. verticillata*. The F3 fraction, with fewer bands on the TLC sheet after successive column chromatography, was confirmed as the indole alkaloid Yohimbine. The π-π* transition corresponding absorption peak at λmax 234 nm in the UV-Vis spectra indicates the presence of conjugated double bonds or aromatic rings in the molecule (Figure 1b). The FTIR

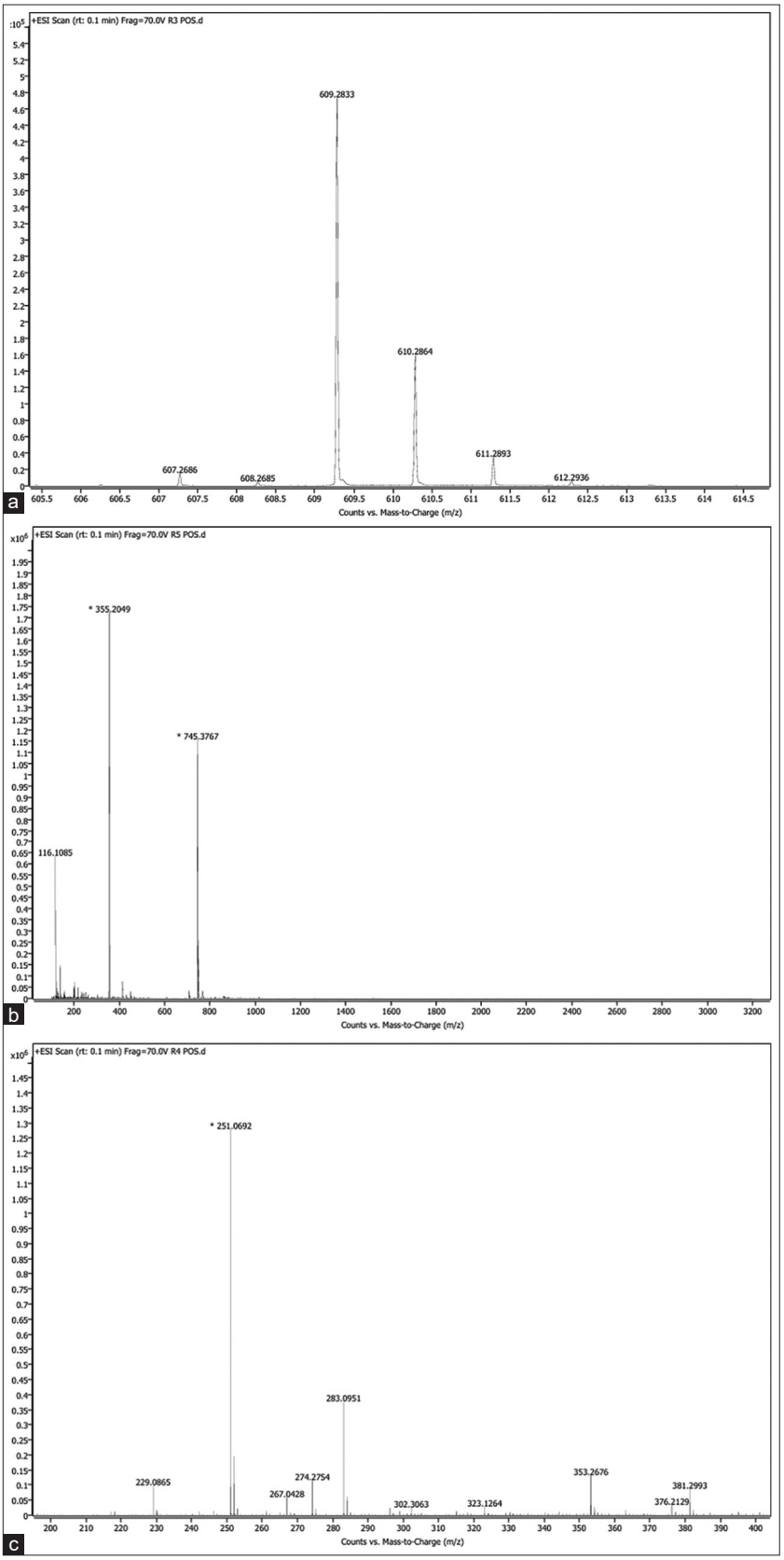

Figure 3: LC-MS spectra of a) Reserpine, b) Yohimbine and c) Seselin

spectrum showed a peak at 3509.81 cm^{-1} corresponding to the N-H stretching vibration. The peaks at 3158.83, 2938.98, and 2887.88 correspond to C-H stretching vibrations. The peak at 1715.37 cm^{-1} is attributed to a stretching vibration of the C=O group. The peak at 1435.74 showed a bending attribution to the C-H bond. The peaks at 1294, 1199.51, 1164.79, 1143.58, 1054.87, and 1023.05 are due to C-O stretching. The peaks at 696.17 and 650.85 cm^{-1} show C=C bending (Figure 2b). In the LC-MS positive-ion mode, the observed peaks at 123.06, 217, and 745.38 are identified as fragmentation peaks. The peak at 355.20 was assigned to the molecular ion peak (M^+). The LC-MS shows a dominant

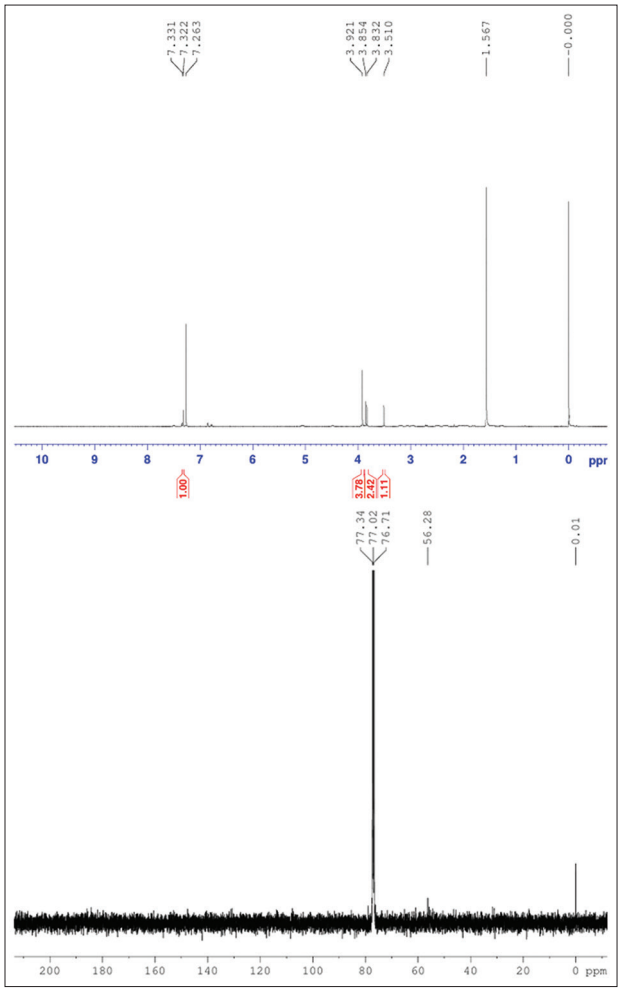

Figure 4: 1H NMR and 13C NMR of Reserpine

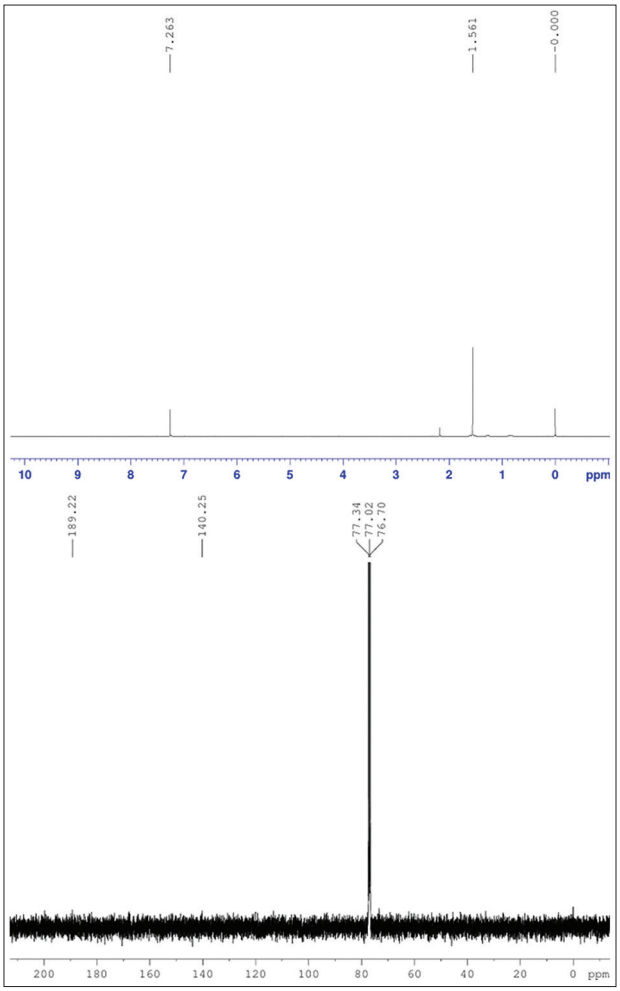

Figure 5: 1H NMR and 13C NMR of Yohimbine

ion at m/z 355.2096, corresponding to the protonated molecular ion $[M+H]^+$. This gives a neutral molecular mass of approximately 354.20 (Figure 3b).

^{13}C-NMR spectra provide structural evidence confirming the carbon framework of Yohimbine. The spectrum is characterized by a high-field carbonyl peak downfield at δ 175.8 ppm (noted in the threshold range near 189.22 ppm), which corresponds specifically to the ester carbonyl carbon (-$COOCH_3$) of the carbomethoxy group. The aromatic and olefinic carbons of the indole core system resonate further upfield in the δ 110-140 ppm region, while the triplet splitting pattern around δ 77.02 ppm clearly marks the standard carbon signal of the $CDCl_3$ solvent. Additionally, the dense cluster of aliphatic signals spanning from δ 21 ppm to 67 ppm resolves the complex skeleton of methylene (CH_2) and methine (CH) carbons inherent to the fused yohimbane ring system, including the distinctive oxygen-bearing C-17 carbon and the ester-bound methoxy methyl carbon (-OCH_3) shifting around δ 52 ppm (Figure 5).

The ^{1}H-NMR spectrum provides structural profiling of yohimbine, a classic indole alkaloid. The spectrum features a distinct downfield aromatic region, typically spanning δ 7.0-7.5 ppm, corresponding to the four proton signals of the fused benzoring system within the indole core. A prominent residual solvent peak for $CDCl_3$ is explicitly recorded at δ 7.263 ppm. Moving upfield, the multiplex of signals distributed through the aliphatic region (δ1.5 ppm to 4.0 ppm) reflects the complex, interconnected stereocenters and methylene/methine protons of the fused yohimbane ring skeleton. This includes highly diagnostic shifts for the carbomethoxy methyl group (-$COOCH_3$) appearing as a sharp singlet typically around δ 3.7-3.8 ppm, and the oxygenated methine proton (H-17) adjacent to the hydroxyl group located near δ 4.2 ppm, confirming the functional architecture of yohimbine (Figure 5).

Compound 3: Seselin

The acetone fraction of *R. verticillata* methanolic stem extract confirmed the presence of coumarin compound, seselin. The presence of conjugated double bonds or aromatic rings in the molecule was indicated by the absorption peak at λmax 292 nm in the UV-Vis spectrum of seselin, corresponding to the π-π* transition (Figure 1c). The FTIR spectrum showed peaks at 3773.04 and 3423.99 cm^{-1}, corresponding to O-H stretching vibrations. The C-H bond stretching vibrations observed in the peaks are 2976.59 and 2932.23. The peaks at 1630.52 and 1590.98 correspond to C=C bond stretching vibrations. The peaks observed at 1437.67 and 1403.92 were attributed to C-O bond stretching. The C-H bending vibration was observed at 731.85 (Figure 2c). The LC-MS spectroscopy detected

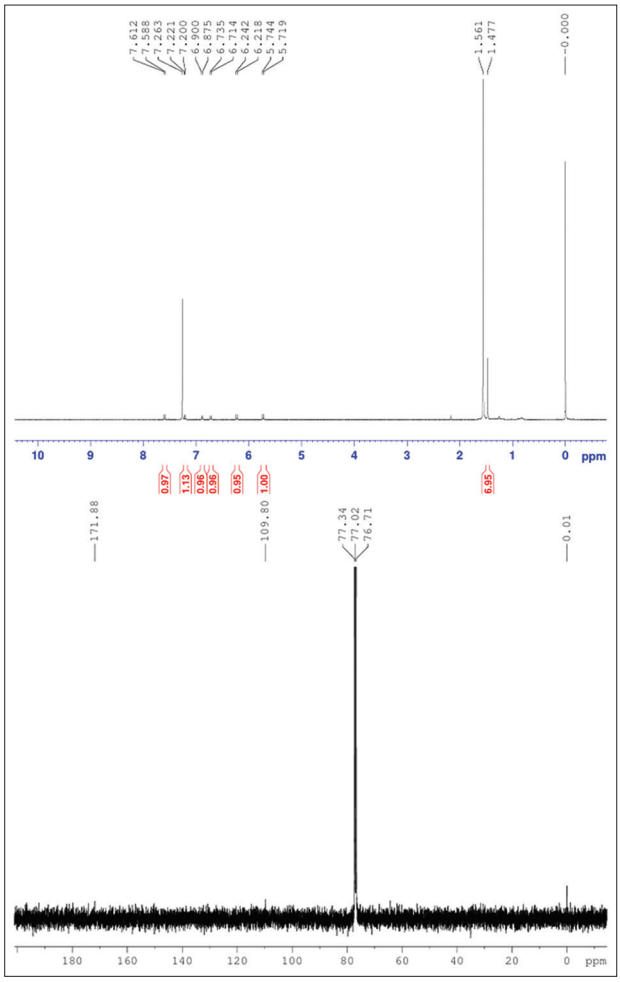

Figure 6: 1H NMR and 13C NMR of Seselin

peaks at 267.04, 274.27, 283.09, 229.085, 302.30, 323.12, 353.26, 376.21 and 381.29, which were symbolised as fragmentation peaks and the peak at 229.08 as molecular ion peaks (M+), and this was conducted in positive ionisation mode (Figure 3c).

^{13}C-NMR spectrum recorded using a power-gated proton-decoupling sequence (zgpg30) in $CDCl_3$ provides definitive structural confirmation of the carbon skeleton of seselin (an angular pyranocoumarin matching Sample-D's ^{1}H profile). The spectrum features a characteristic downfield carbonyl resonance at δ 171.88 ppm, corresponding to the lactone carbonyl carbon (C-2) of the central pyrone ring. The aromatic and olefinic carbons of the core coumarin skeleton and the fused dimethylpyran ring resolve cleanly across the δ100-160 ppm region, anchored by a prominent peak at δ 109.80 ppm, which represents one of the electron-rich aromatic methine carbons. The standard triplet pattern of the $CDCl_3$ solvent is clearly present at δ 77.02 ppm. Finally, the upfield region (δ 20-30 ppm) captures the sharp carbon signal for the equivalent gem-dimethyl groups attached to the quaternary pyran carbon (C-2'), completing the spectroscopic layout of the compound (Figure 6).

^{1}H-NMR spectral data strongly align with the structural profile of seselin, a linear angular pyranocoumarin. The spectrum displays characteristic downfield aromatic and olefinic proton signals between δ 5.71 ppm and 7.61 ppm, corresponding to the coumarin lactone double bond and the fused benzene/pyran ring system. Specifically, the distinct doublets near δ 6.23 ppm and 7.60 ppm correspond to the cis-coupled protons of the pyrone ring (H-3 and H-4), while the doublets near δ 5.73 ppm and 6.89 ppm represent the olefinic protons of the fused dimethylpyran ring. A residual $CDCl_3$ solvent peak is visible at δ 7.26 ppm, and a highly intense singlet with an integration of ~6H, appearing upfield at δ 1.48 ppm, cleanly confirms the presence of the two equivalent gem-dimethyl groups unique to the pyran ring structure (Figure 6).

Discussion

R. verticillata is an indigenous medicinal plant in China and is notable for its ability to produce a wide range of indole-based heterocyclic alkaloids of pharmacological relevance (Gao *et al.,* 2015). The identification of two indole alkaloids and a coumarin compound in *R. verticillata* was confirmed through detailed spectroscopic analysis. Yohimbine and reserpine are pharmacologically important indole alkaloids, known for their central nervous system activity, including reported antipsychotic and antihypertensive properties (Hong *et al.,* 2012; Kumar *et al.,* 2016). Reserpine acts by completely disrupting the vesicular monoamine transporter, resulting in the depletion of neurotransmitters, including norepinephrine, serotonin, and dopamine, which accounts for its antihypertensive and sedative effects. Seselin is a naturally occurring coumarin compound isolated from plant extracts and has diverse pharmacological properties. Seselin and its derivatives exhibit biological activities including antioxidant, antimicrobial, anticancer and anti-inflammatory effects. It has also been examined as a lead compound in drug development due to its cytotoxic activity against specific cancer cell lines.

The LC–MS analysis of reserpine showed a molecular ion peak at m/z 609 $[M+H]^{+}$, which correlates well with reported data (Hong *et al.,* 2010). The structural identity of reserpine was further confirmed by ^{1}H and ^{13}C NMR spectra, consistent with earlier studies (Arambewela & Madawela, 2001; Hong *et al.,* 2012). The FTIR spectrum exhibited characteristic absorption bands at approximately 3500, 2950, and 1610 cm^{-1}, aligning with previously reported spectral features (Timmins & Court, 1974). The presence of yohimbine was established by LC–MS, which showed a protonated molecular ion peak at m/z 355 $[M+H]^{+}$+, consistent with earlier reports (Hong *et al.,* 2010; Maurya *et al.,* 2013). The UV and FTIR spectral data showed characteristic absorption at around 230 nm and functional group peaks near 2950 and 1715 cm^{-1}, respectively, which are in accordance with previously reported values (Timmins & Court, 1974). Other classes of compounds, including coumarins and acridone alkaloids, have also been reported. The presence of seselin was confirmed by 1H and 13C NMR analyses, consistent with previous findings (Cazal *et al.,* 2009; Hong *et al.,* 2012). Previous studies have also reported the isolation of yohimbine and reserpine from different parts of Rauvolfia species, including the stem (Akinloye & Court, 1981; Mahalakshmi *et al.,* 2019), supporting its widespread occurrence within the genus.

Conclusion

In conclusion, this study successfully established an efficient extraction and purification workflow to isolate high-value bioactive secondary metabolites from the rare medicinal shrub *R. verticillata*. Utilizing column chromatography guided by Thin-Layer Chromatography (TLC), three major phytochemicals were successfully isolated from the methanolic stem extract in their homogenous forms. The structural identity and integrity of the isolated compounds were examined by extraction and extensive spectroscopic analysis; the resulting spectral data showed strong agreement with previous reports. These findings support the potential of *R. verticillata* as a useful source of bioactive compounds and confirm the presence of pharmacologically active metabolites. This study provides a strong basis for further research to fully determine the therapeutic effectiveness of these compounds, including pharmacological evaluation, toxicity assessment, and in vivo validation. In conclusion, this study successfully established an efficient extraction and purification workflow to isolate high-value bioactive secondary metabolites from the rare medicinal shrub *R. verticillata*. To transition these laboratory findings into viable therapeutic applications, immediate future efforts will focus on profiling the safety and toxicity of these isolated compounds and conducting rigorous *in vivo* pharmacological assays to fully evaluate their mechanisms of action and therapeutic efficacy.

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Author contributions

Nisha Ayyappan: Planning of work, methodology, writing original manuscript; Ramasubbu Raju: Planning of work, project administration, review and editing of original manuscript; E. G. Wesely Jebasing Devairakkam: project administration, review and editing of original manuscript.

References

Akinloye, B. A., & Court, W. E. (1981). The alkaloids of *Rauwolfia volkensii*. *Journal of Ethnopharmacology, 4*(1), 99-109. https://doi.org/10.1016/0378-8741(81)90022-2

Arambewela, L. S. R., & Madawela G. (2001). Alkaloids from *Rauvolfia camescens*. *Pharmaceutical Biology, 39*(3), 239-240. https://doi.org/10.1076/phbi.39.3.239.5923

Ayyappan, N., Raju, R., & Wesely Jebasing Devairakkam, E. G. (2025). Ethnobotany, bioactive compounds and therapeutical values of *Rauvolfia tetraphylla* L.- A review. *Current Pharmacology Reports, 11*, 21. https://doi.org/10.1007/s40495-025-00400-7

Cazal, C. de M., Domingues, V. de C., Batalhão, J. R., Bueno, O. C., Filho, E. R., da Silva, M. F., Vieira, P. C., & Fernandes, J. B. (2009). Isolation of xanthyletin, an inhibitor of ants' symbiotic fungus, by high-speed counter-current chromatography. *Journal of Chromatography A, 1216*(19), 4307-4312. https://doi.org/10.1016/j.chroma.2009.02.066

Chen, W., Liang, W., Li, A., & Ma, J. (2019). Characterization of the complete plastid genome of *Rauvolfia verticillata* (Apocynaceae), with its phylogenetic analysis. *Mitochondrial DNA Part B, 4*(2), 4190-4191. https://doi.org/10.1080/23802359.2019.1693287

Dey, A., & Pandey, D. K. (2014). HPTLC detection of altitudinal variation of the potential antivenom stigmasterol in different populations of the tropical ethnic antidote *Rauvolfia serpentina. Asian Pacific Journal of Tropical Medicine, 7*(S1), S540-S545. https://doi.org/10.1016/S1995-7645(14)60287-X

Gao, Y., Wang, F., Zhou, D.-S., Li, Y., & Liu, J.-K. (2011). Three new indole alkaloids from *Rauvolfia yunnanensis*. *Natural Products and Bioprospecting, 1*, 104-107. https://doi.org/10.1007/s13659-011-0023-7

Gao, Y., Yu, A.-L., Li, G.-T., Hai, P., Li, Y., Liu, J.-K., & Wang, F. (2015). Hexacyclic monoterpenoid indole alkaloids from *Rauvolfia vericillata*. *Fitoterapia, 107*, 44-48. https://doi.org/10.1016/j.fitote.2015.10.004

Hong, B., Cheng, W., Wu, J., & Zhao, C. (2010). Screening and identification of many of the compounds present in *Rauvolfia verticillata* by use of High-Pressure LC and quadrupole TOF MS. *Chromatographia, 72*, 841-847. https://doi.org/10.1365/s10337-010-1760-1

Hong, B., Gao, J., Wu, J., & Zhao, C. (2012). Chemical constituents from *Rauvolfia verticillata* and bioactivities research. *Chemistry of Natural Compounds, 48*, 276-280. https://doi.org/10.1007/s10600-012-0220-6

Hong, B., Su, Z., Zhang, C., Yang, Y., Guo, Y., Li, W., & Kong, T. A. (2016). Reserpine inhibit the JB6 P+ cell transformation through epigenetic reactivation of Nrf2-mediated anti-oxidative stress pathway. *The AAPS Journal, 18*, 659-669. https://doi.org/10.1208/s12248-016-9901-6

Katzung, B. G. (2021). *Basic and clinical pharmacology*. (15th ed.). McGraw-Hill.

Kumar, S., Singh, A., Bajpai, V., Srivastava, M., Singh, P. B., Ojha, S., & Kumar, B. (2016). Simultaneous determination of bioactive monoterpene indole alkaloids in ethanolic extract of seven *Rauvolfia* Species using UHPLC with hybrid triple quadrupole linear ion trap mass spectrometry. *Phytochemical Analysis, 27*(5), 296-303. https://doi.org/10.1002/pca.2631

Kumari, I., Madhusudan, S., Walia, B., & Chaudhary, G. (2021). *Rauwolfia serpentine* (Sarpagandha): A review based upon phytochemistry and ayurvedic uses. *International Journal of Current Research, 13*, 16727-16734.

Mahalakshmi, S. N., Achala, H. G., Ramyashree, K. R., & Kekuda, T. R. P. (2019). *Rauvolfia tetraphylla* L. (Apocynaceae)- A comprehensive review on its ethnobotanical uses, phytochemistry and pharmacological activities. *International Journal of Pharmacy and Biological Sciences, 9*(2), 664-682. https://doi.org/10.21276/ijpbs.2019.9.2.81

Maurya, A., Gupta, S., & Srivastava, K. S. (2013). Large-scale separation of antipsychotic alkaloids from *Rauvolfia tetraphylla* L. by pH-zone-refining fast centrifugal partition chromatography. *Journal of Separation Science, 36*(2), 407-413. https://doi.org/10.1002/jssc.201200273

Mousavi, S. H., Davari, A. S., Iranshahi, M., Sabouri-Rad, S., & Najaran, Z. T. (2015). Comparative analysis of the cytotoxic effect of 7-prenyloxycoumarin compounds and herniarin on MCF-7 cell line. *Avicenna Journal of Phytomedicine, 5*(6), 520-530. https://doi.org/10.22038/ajp.2015.4355

Rohela, G. K., Bylla, P., Korra, R., & Reuben, C. (2016). Phytochemical screening and antimicrobial activity of leaf, stem, root and their callus extracts in *Rauwolfia tetraphylla*. *International Journal of Agriculture and Biology, 18*(3), 521-528.

Saranya, S., Ramasubbu, R., Prasannan, P., & Surendran, A. (2021).

A comprehensive review on ethnobotany, phytochemistry and pharmacology of *Rauvolfia* L. (Apocynaceae). *The Botanical Review, 87*(3), 311-376. https://doi.org/10.1007/s12229-021-09262-2

Sulaiman, C. T., Jyothi, C. K., Unnithan, J. K., Prabhukumar, K. M., & Balachandran, I. (2020). Identification of suitable substitute for Sarpagandha (*Rauvolfia serpentina* (L.) Benth. ex Kurz) by phytochemical and Pharmacological evaluation. *Beni-Suef University Journal Basic and Applied Sciences, 9*, 42. https://doi.org/10.1186/s43088-020-00069-5

Tam, S. W., Worcel, M., & Wyllie, M. (2001). Yohimbine: a clinical review. *Pharmacology & Therapeutics, 91*(3), 215-243. https://doi.org/10.1016/S0163-7258(01)00156-5

Timmins, P., & Court, W. E. (1974). Root alkaloids of *Rauwolfia oreogiton*. *Planta Medica, 26*(6), 170-173. https://doi.org/10.1055/s-0028-1097985

Venugopala, K. N., Rashmi, V., & Odhav, B. (2013). Review on natural coumarin lead compounds for their pharmacological activity. *Biomed Research International, 2013*(1), 963248. https://doi.org/10.1155/2013/963248