STM JOURNALS

Emerging Trends in
Metabolites

Volume 1, Issue 2, 2024
July–December
DOI (Journal): 10.37591/ETM

https://journals.stmjournals.com/etm

Review ETM

Rauwolfia Serpentina: A Comprehensive Phytochemical Study of Its Bioactive Metabolites

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Abstract

Rauwolfia Serpentina is an important medicinal plant used in Ayurveda, Unani, and folk medicine system of medicine. This plant grew as a bush in company of other plants as an evergreen structure. It is commonly known as 'Sarpaghanda'. in Ayurvedic and Unani medicine system. It is safe and effective for the treatment of snakebites and various types of disease like malaria, breast cancer, prostate cancer, high blood pressure, insomnia, epilepsy, and traumas. Its therapeutic properties are associated with the presence of various phytochemical compounds such as alkaloids, phenols/polyphenols, saponins, tannins, yohimbine and reserpine. Additionally, these bioactive molecules possess remarkable hypoglycemic, anti-diarrheal, anti-bacterial, and anxiolytic properties. R. serpentina known as the Indian snakeroot in ayurvedic system of medicine. It has been used as a very important distinctive medicinal shrub for a long time. The different parts of R. serpentina have bioactive phytochemicals beneficial in controlling human disease like insomnia, insanity, anxiety, sleeplessness, high blood pressure and various other cardiovascular disease affecting human beings. However, the concentration of different phytochemicals varies in root, stem, bark, berry, and leaves of R. serpentina. It may prove to be an "all in one" cure for various human ailments. It has now been cultivated throughout the India and has a wide application in the traditional system of homeopathic medicine also for curing human diseases. It is available as powder, tablet, capsule and liquid syrups and alcoholic dilutions in various herbal formulations available in the market belonging to this plant.

Keywords: Alkaloids, anti-diarrheal, anti-bacterial anxiolytic, anti-hypertensive activity, *Rauwolfia serpentina*

INTRODUCTION

In the present time emergence of new and fatal human disease, research based on studies of Indian customary medicine systems like Ayurveda, Siddha and Unani have gained momentum [1]. The medicinal alternatives provided by these ancient medicinal systems have benefitted not only the Indian sub-continent but also the whole world population. In post COVID -19 era, many developed countries have also shown their faith in clinical benefits of these alternative drugs including U.S.A., U.K., Germany, Japan, etc. [2]. In India, the masses have been using these formulations from time unknown in form of spices, condiments, flavoring agents and home-made remedies [3]. Karl von Linnaeus originally described Rauwolfia in 1753 in Species Plantarum. This is a bush growing in gathering of plants as an evergreen structure, commonly known as 'Sarpaghanda' as recorded in Ayurvedic and Unani medicine system. The Sarpaghanda has been mentioned and used in Indian medicinal system from the time of Charaka. In India, this species has been found growing naturally at an altitude 1300 to 1400m in the foothills and slopes of Himalaya. It grew sparsely in a few zones of Punjab, Assam,

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Received Date: August 20, 2024
Accepted Date: September 02, 2024
Published Date: September 03, 2024

Citation: Mukesh Chander. *Rauwolfia Serpentina:* A Comprehensive Phytochemical Study of Its Bioactive Metabolites. Emerging Trends in Metabolites. 2024; 1(2): 25–50p.

Meghalaya, Sikkim, J&K, Uttarakhand, Western Ghats, and Andaman [4]. The father of the nation, Mahatma Gandhi has used *Rauwolfia* based beverage to reduce stress and anxiety [5]. *Rauwolfia serpentina* is an evergreen bush belonging to family – Apocynaceae. It has been reported that the genus *Rauwolfia* has more than 100 species. These species are found to grow naturally in tropical and subtropical regions throughout the world, including Europe, Africa, Asia, Australia and Central and South America. *R. serpentina* is local to the forests with moist and humid climate located in southeast Asia comprising of India, Pakistan, Burma, Myanmar, Bangladesh, and Malaysia. The plant has light green leaves which are 3.5 to 5.0 cm wide (Figure 1) [6]. The height of the *R. serpentina is* in the range of 60cm to 90cm. The leaves are curved or lanceolate formed and are arranged in whorls or clusters of 3 to 5 leaves. The plant has numerous sparkling, dark or purple, round fruits of around 0.5cm diameter. It likewise has little pink or white blossoms Figure 1 (a) and (b). The plant has a conspicuous tuberous, delicate taproot that may achieve length in the range of 30 and 50 cm and the diameter in the range of 1.2 and 2.5 cm [7].

The aliquots obtained from different parts of *R. serpentina* essentially contain a variety of bioactive molecules. These include Yohimbine, Serpentinine, Reserpine, Ajmaline, Rescinnamine, and Deserpidine etc. This shrub has been reported as a significant restorative plant having some unique remedial properties. In the past, the bioactive extracts obtained from different parts of this plant have been used for treatment of various human disease but lacks proper pharmacological studies with scientific backup. The disease curing potential of *R. serpentina* may be attributed to the presence of one or all of the bioactive compounds namely, alkaloids, sugars, flavonoids, glycosides, phenols, tannin, saponins, sterols, phlobatannins, saps and terpenes.

Figure 1. Plant of *Rauwolfia serpentina.* (a) *Rauwolfia Serpentina* plant, (b) Root of *R. serpentina.*

Transverse section of *Rauwolfia*

The transverse section of the *Rauwolfia* root shows cork, which is isolated, into distinct zones (2 to 8) and comprises of one to seven cell layers of more modest and radially smaller, suberized, non-lignified cells exchanging with bigger radially more extensive, lignified cells one to three layers thick. The phelloderm is made from around ten to twelve layers of digressively extended to isodiametric, cellulosic parenchymatous cells. Cells parenchyma substitute with more extensive medullary beams made from enormous cells and normally two to four cells wide. Xylem is wide, altogether lignified and ordinarily shows multiple yearly rings. Medullary beams comprise of starch grains and substitute with optional xylem comprising of vessels, tracheids, filaments and parenchymatous tissue (Figure 2). The xylem vessels are thoroughly lignified and have pitted thickening. Vessels are little as contrast with different species.

Classification & History

'Father of Indian medicine' and 'Father of Plastic Surgery', Sushtra introduced this plant for formulate and found surgical procedure. It reports of medicinal use of this species has been reported way back to pre-vedic era, as a medication to cure poisoning due to snakebites and fever of unknown origin and high grade [8]. The ancient literature (Charak Samhita -1000 to 800 Before Christ) reported that extracts of this plant cured skin allergies due to insect stings (Table 1).

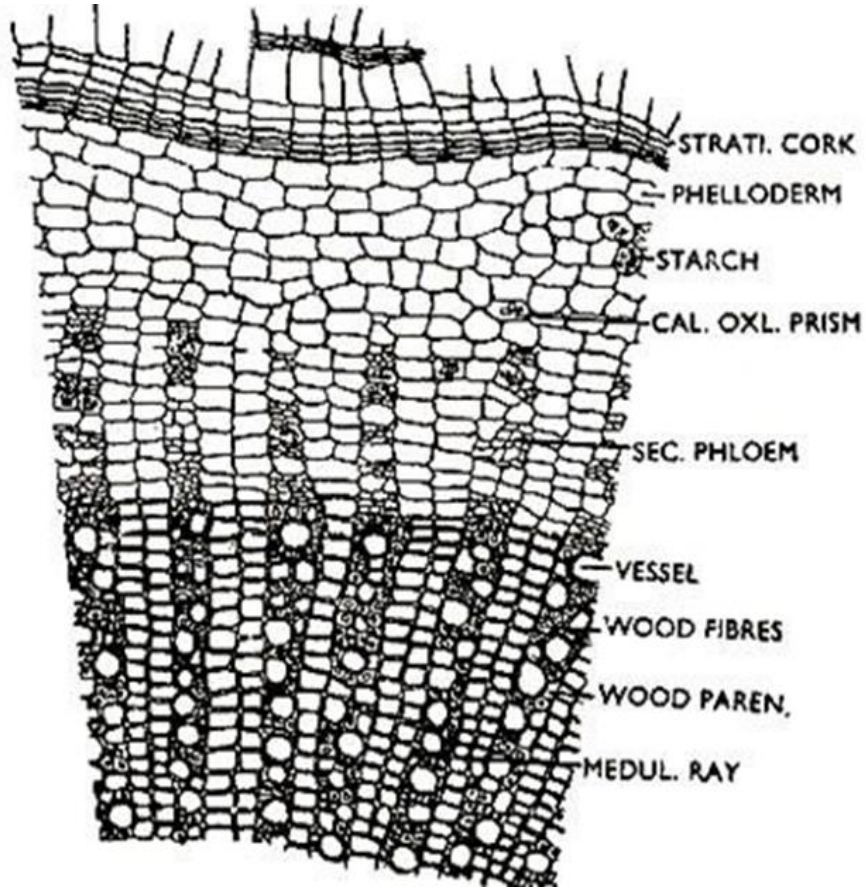


Figure 2. Transverse section of *Rauwolfia.*

Table 1. Classification of *Rauwolfia serpentina*.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliophyta
Order	Gentianales
Family	Apocynaceae
Genus	*Rauwolfia*
Species	*Serpentina*

In India, the root of this plant has been utilized for many years. The clinical report of English distribution propose that this plant was utilized in treatment of 50 cases of hypertension. The species of *R. serpentina* has found great application in Indian framework (Ayurveda, Siddha, Unani) and Western system of medicines [10].

Rauwolfia serpentina was utilized in the society of medication in India for quite a long time to treat a variety of illnesses, including snakebites furthermore, malaria, febrile conditions, intestinal sickness, stomach pain, and it was utilized as an uterine energizer, febrifuge, and solution for craziness. The

variety *Rauwolfia* was named out of appreciation for the sixteenth century German doctor Dr. Leonhard Rauwolf, who examined plants while being in India. *R. serpentina* was chosen for study due to the presence of its long, tightening, snake-like roots. The Indian doctor, Rustom Jal Vakil, was responsible for introducing the *Rauwolfia* with Western medication. From 1939 to 1949, he gathered data of patients cured with *R. serpentina* treatment. In 1949, he wrote a lengthy report entitled 'The Antihypertensive Properties of Rauwolfia serpentina' for the British Medical Journal. He introduced his outcomes from treating 50 hypertensive patients using the root extract of *R. serpentina* (Table 2). The patient response was exceptional, increasing the use of rauwolfia in the treatment of hypertension by 90%. More than 100 scientific papers endorsing Vakil's work were published all over the world. In the Atharva Veda, verses gave evidence of traditional use of medicinal plants for more than 300 years .

REVIEW OF LITERATURE

The practice of using roots extract of the plant *R. serpentina* as the remedy for curing various disease like epilepsy, anxiety, high blood pressure (Hypertension), insomnia and mental disorder, gastrointestinal problem, schizophrenia, traumas, has been done in the Ayurvedic system of medicines [11, 12, 13]. The plant roots extract can treat diseases like dizziness, oligomenorrhea, dysmenorrhea, amenorrhea, like abnormalities in the Siddha medicine [14] (Table 3).

Table 2. Various vernacular names of *Rauwolfia* (Adapted from www.ecoplanet.in/herbs) [11].

S.N.	Language	Common Name
1	Hindi	Chandrabhaga, Chota-chand, Sarpaganda
2	English	*Rauwolfia* and Indian snakeroot
3	Latin	*Rauwolfia serpentina*
4	Sanskrit	Sarpaghanda
5	Tamil	Chevanamalpodi,Sarpaghanda
6	Kannada	Keramaddinagadi
7	Telugu	Patalaguni,Patalagandha,Sarpagandha
8	Malayalam	Svapavalporiyam,Churannavilpori
9	Marathi	Harkaya,Harki,Hadaki,Adakai
10	Assamese	Arachoritita
11	Bengali	Chandra
12	Chinese	Lu fu mu

Table 3. Ayurvedic pharmacodynamic properties of *R. serpentina* [15].

S.N.	Property	Characteristic
1.	Rasa	Tikta
2.	Guna	Ruksha
3.	Veerya	Ushna/Agney
4.	Vipaka	Katu

Chemical Composition

Rauwolfia contains three types of phytochemical/ alkaloids:

1. Weakly basic indole alkaloids.
2. Intermediate basicity of indoline alkaloids.
3. Strong Andronium bases alkaloids [16].

The plant *Rauwolfia* contains the indole alkaloids, which are the most important alkaloids. These alkaloids are nitrogenous compounds derived from the amino acid 'tryptophan'. Mostly all parts of plant contain indole alkaloids, including stem and leaves of plant, condensed in the outer cellular zone of the root. Indole alkaloids include ajmalidine, ajmaline, ajmalicine, aricine, yohimbine, yohimbinine, sarpagine, and sarpentine.

Mineral Composition

Number of macro-nutrients and micro-nutrients are present in the *Rauwolfia* plant but calcium is an abundant macro-nutrient [17]. High calcium content presence in these plants benefits in healing the wounds and helps in blood coagulation. Ascorbic acid, riboflavin, thiamine, and niacin are essential sources of the *R. serpentina* (Table 4) [18].

Phytochemical Constituents

The various types of phytochemical compounds and secondary metabolites found in *R. serpentina* include alkaloids, phenols, tannins and flavonoids.

Alkaloids

Alkaloids are a big group of organic particles which contain a heterocyclic nitrogen ring which are consumed by various microorganisms and animals. About 10% of plant species produce alkaloids as secondary metabolites, which provide protection against herbicides and diseases. The synthetic derivatives of these natural alkaloids are utilized as therapeutic specialists' agents for their pain relieving, antispasmodic and bactericidal effects [19, 20]. The alkaloids acquired from the root extract respond directly on focal CNS system and thereby, lower the blood pressure faster as compared to other agents that have been helping in lowering down the blood pressure. It has been reported 0.7–3.0% of all alkaloids are present in plant *R. serpentina* and approximately 0.1% of the reserpine (an indole alkaloid),located in the root of plant. Thus, production of root biomass of this plant could be economical importance [21].

Table 4. Concentration of Micro, Macro element present in *Rauwolfia serpentina* [19].

S.N.	Micro Elements		Macro Elements		Vitamin	
	Name of Micro Element	***Concentration***	***Name of Macro Element***	***Concentration***	***Name of Vitamin***	***Concentration***
1.	Ni^{60}	7.09 ± 0.042	Al	785.7 ± 46.0	Ascorbic	44.03 ± 0.20
2.	Cu^{63}	9.46 ± 0.442	Ca	6515.8 ± 562.3	Riboflavin	0.42 ± 0.12
3.	Zn^{64}	20.23 ± 0.779	Fe	630.7 ± 37.3	Thiamine	0.18 ± 0.02
4.	Sr^{87}	164.50 ± 7.79	K	10256.4 ± 492.1	Niacin	0.02 ± 0.10
5.	Rb^{85}	13.10 ± 7.77	Mg	1157.1 ± 33.6	-	-
6.	-	-	Na	196.3 ± 23.0	-	-
7.	-	-	P	2131.2 ± 159.0	-	-
8.	-	-	S	573.4 ± 26.3	-	-

The different alkaloids found in *R. serpentina* reserpine, ajmalicine, ajmaline, ajmalimine, indobine, deserpidine, indobinine, reserpiline, rescinnamine, rescinnamidine, serpentine, and yohimbine, etc. [22, 23]. Reserpine, yohimbine, serpentine, deserpidine, ajmalycin, and ajmaline are examples of alkaloids used to treat hypertension and breast cancer [24–27].

Reserpine

In 1952, reserpine was first extracted from the root of *Rauwolfia crude*, reserpine has been found to be purely crystalline single alkaloid [28]. It has a melting point 26 to 27° C and contain pentacyclic nucleus. Weak tertiary base of the roots in the oleoresin zone of the roots and helpful in the management and control of hypertension, cardiovascular abnormalities, and neurological diseases [29, 30]. The antihypertensive properties of *Rauwolfia* have been attributed to reserpine (3,4,5-trimethyl benzoic) and the conjugates of 18-hydroxy yohimbine and utilized primarily as a tranquillizer (Figure 3) [5, 31, 32].

The antihypertensive activities of reserpine are because of its depressant activity on central and peripheral nervous system by restricting the activity of catecholamine reservoirs present in the nerve cell. This decreases the storage of catecholamines and serotonin in nerve cells. It also affects the

autonomic nervous system, reduces neurotransmitter levels in adrenergic neurons, and increases the parasympathetic system [33, 34]. These substances get engaged in controlling pulse, heart constriction and peripheral resistance, helping in sedation and bringing down the stress and nervous system movement. Reserpine releases 5-hydroxytryptamine (5-HT) from total tissue reservoirs, increasing urinary output [35]. Reserpine is currently being used as a tool in physiologic investigations of body functions and in pharmacological examinations.

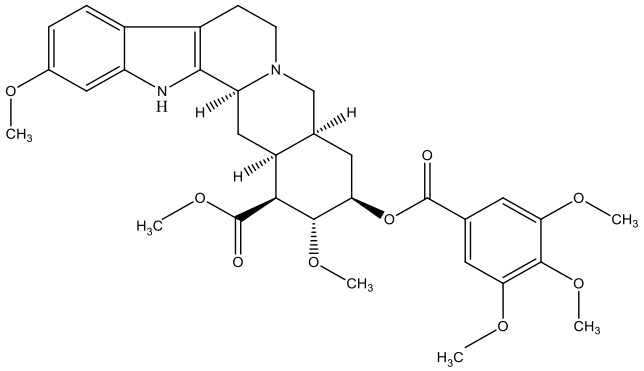


Figure 3. Structure of Reserpine.

Ajmaline

In year 1931, Ajmaline was first isolated by Salimuzzaman Siddiqui. One of the most distinguished specialists of Unani medicine in South Asia, Hakim Ajmal Khan, named it as Ajmaline [36, 37]. Derived from the *R. serpentina* root extract, ajmaline has been branded as the best 'antiarrhythmic agent'. It has an important role in diagnosis of Brugada Syndrome (congenital heart disease), and patients with this disease [38]. These drugs are divided into four groups based on their mechanism of action: sodium channel blockers (SCB), beta adrenergic blockers (BAB), repolarization prolongation, and calcium channel blockers. For diagnostic purpose, Ajmaline is a blocker of sodium channel in cells which shows instant activity when given intravenously (Figure 4) [39, 40]. It has been reported to stimulate breath and intestinal movements in a way like that of serpentine [41].

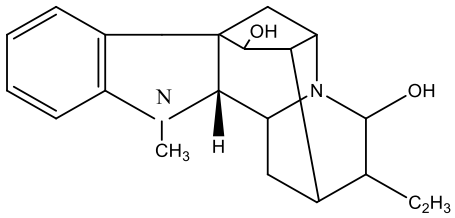


Figure 4. Structure of Ajmaline.

Serpentine

Serpentine is a type 2 topoisomerase inhibitor and exhibits antipsychotic properties. The enzyme PER in the vacuole catalyzes the oxidation of ajmalycin to serpentine by the bisindole alkaloid (Figure 5) [42].

Rescinnamine

Rescinnamine, an ester alkaloid of alsero xylon in types of *Rauwolfia*; related synthetically and pharmacologically to reserpine with same uses. Rescinnamine was first clinically tested for its antihypertensive activity in 1950. Clinically, it is a weaker alkaloid than reserpine and less effective in lowering blood pressure [43]. Recinamine inhibits peptide dipeptidase, an angiotensin-converting enzyme that converts angiotensin I to angiotensin II, a vasoconstrictor that stimulates the adrenal cortex to secrete aldosterone. Firstly, it represses the Angiotensin Converting Enzyme (ACE) and then, at that point, it blocks angiotensin I conversion to angiotensin II. Hindrance of ACE outcomes in diminished

plasma angiotensin II. As angiotensin II vasoconstrictor and a negative input for renin action, it lowers blood pressure, as well as stimulate baroreceptor reflex systems, which at last outcomes in diminished vasopressor movement and, aldosterone secretion (Figure 6).

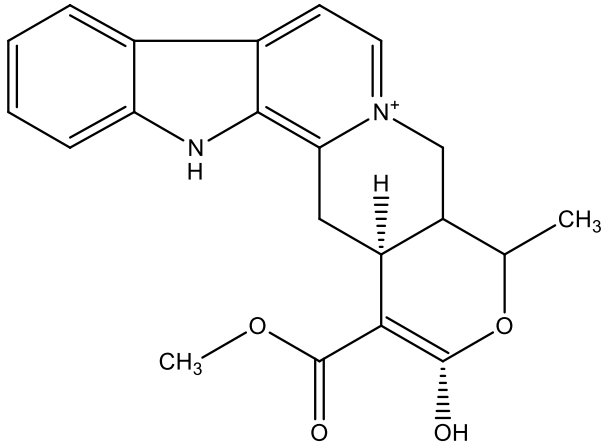


Figure 5. Structure of Serpentine.

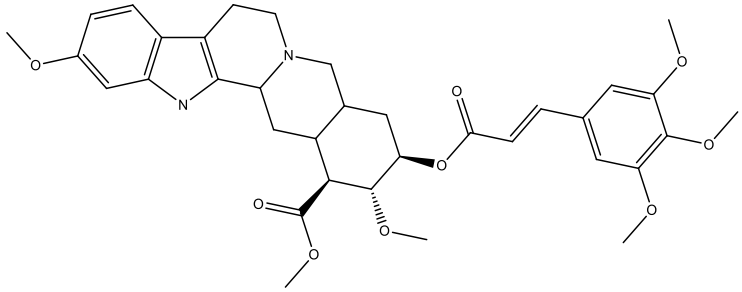


Figure 6. Structure of Rescinnamine.

Deserpidine

Deserpidine is an ester alkaloid which is derived from *Rauwolfia*. Deserpidine differs from reserpine in the absence of a methoxy group at C-11 derived from reserpine. It is often used for its antipsychotic and antihypertensive properties (Figure 7). It has been reported to decrease blood pressure by controlling various neural pathways, further reducing psychotic behavior. It represses the activity of angiotensin converting enzyme and angiotensin I and II [44].

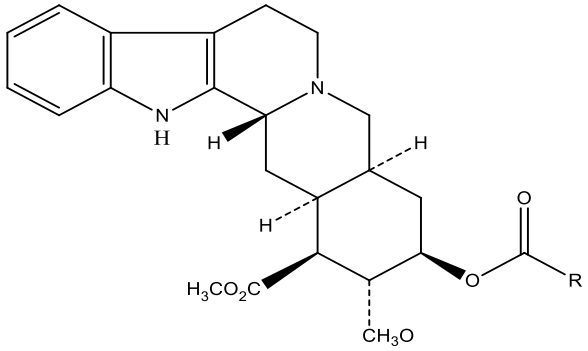
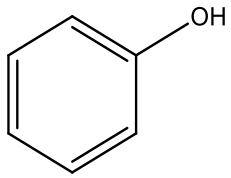


Figure 7. Structure of Deserpidine.

Phenols

Phenols are the secondary plant metabolites broadly circulated in the plant kingdom essentially in spices, bushes, vegetables, and trees [48, 49]. The presence of phenols due to their poisonous nature

can be used for the development and advancement of different pest, microbes, and pathogens [50] R.S. It has therapeutic properties, such as diuretics and laxative (Figure 8). The presence of phenolic compounds suggests potential antimicrobial activity, although further systematic studies are needed.



Phenol
C_6H_6O
(Properties, Uses)

Figure 8. Structure of Phenol.

Tannins

The presence of gallic acid and digallic acid tannins provide antioxidant properties (Figure 9) [53]. Tannins have been found to be excellent healers, they disinfect and start healing wounds and inflamed mucous membranes. In Southeastern part of India, *R. serpentina is* used in treating many disorders by traditional medicine healers [54, 55].

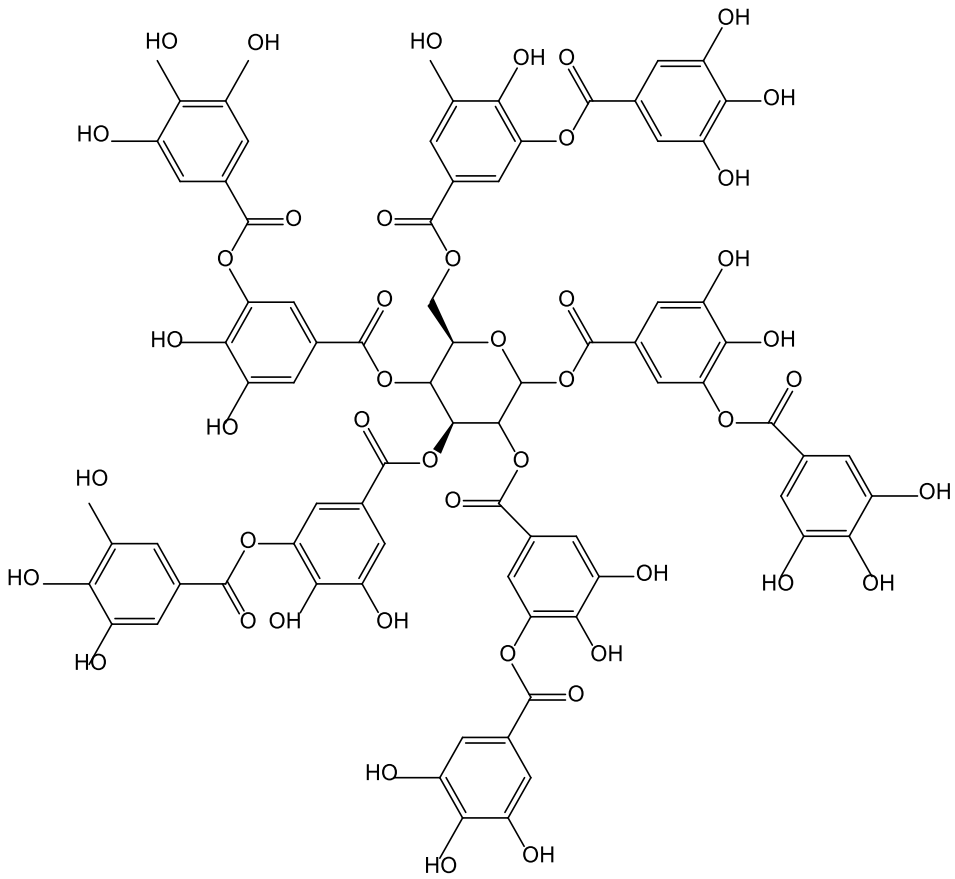


Figure 9. Structure of Tannin.

Yohimbine

Yohimbine is utilized as an alpha-adrenergic adversary and alpha-blocker in the veins for the cure of erectic brokenness. It enlarges the veins and increase the blood flow in the penis, that assist in improving erectile function [45, 46]. Yohimbine has been used to treat diabetes by targeting polymorphisms of the α2A-adrenergic receptor gene in animals and humans. Blocking these muscles relaxes muscle spasms and lowers blood pressure (Figure 10). This process is accompanied by an increase in the levels of specific chemicals in the body, causing the pupils to dilate [47].

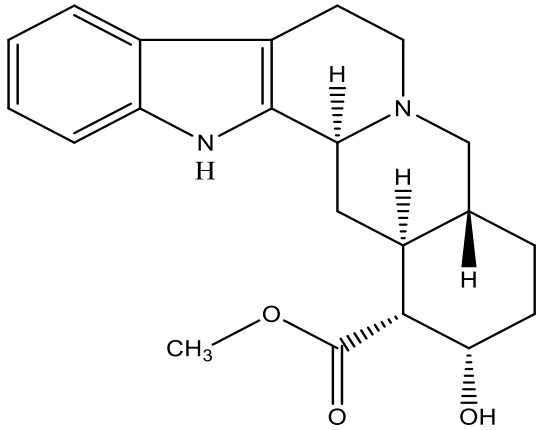


Figure 10. Structure of Yohimbine.

Flavonoids

Flavonoids are powerful water-soluble antioxidants and scavenges free radicals to protect oxidative cell damage, thus showing anti-cancerous activity [56, 57]. Flavonoids in intestinal tract likewise bring down the danger of heart problems or illness. As antioxidants flavonoids of herbal medicine, it produces anti-inflammatory action [58, 59].

Saponins

Saponins are glycosides derived from triterpene sterols that have been identified in more than 70 plant groups. Some of the attributes of saponins involved the froth formation in aqueous solution, hemolytic movement, cholesterol restricting properties and bitterness [60]. Saponins can cause clumps of red blood cells. To restrict bleeding and in healing wounds, studies suggested the use of high saponin content of *Rauwolfia serpentina* [55, 61].

Ajmalicine

Alkaloid, ajmalicine, found its numerous applications in the cure and management of circulatory sicknesses, particularly in giving relief to ordinary cerebral blood flow. It influences the capacity of smooth muscle, prevents strokes and brings down blood pressure [22]. Approximately 3500 kg of ajmalicine obtained from either *Rauwolfia* or *Catharanthus* spp. has been reported to be consumed annually by pharmaceutical industries for the manufacturing of drugs used for treatment of circulatory sickness. The biosynthetic pathway for synthesis of ajmalicine begins with geraniol as starting metabolite forming loganin finally transforming to iridodial and irdotrial, which on oxidation changes over loganin into secoloanin. This aide the change of tryptamine to formcorynanthe type nucleus that outcomes in the development of ajmalicine (Figure 11) [24, 62]. The ajmalicine is a derivative of tryptophan which is changed over to tryptamine through secologanin, strictosidine and cathenamine. Decrease of cathenamine to ajmalicine is worked with by NADPH enzyme and tryptophan decarboxylase (TDC). A study has suggested that decarboxylase is key enzyme associated with the combination of ajmalicine in *Rauwolfia* [63].

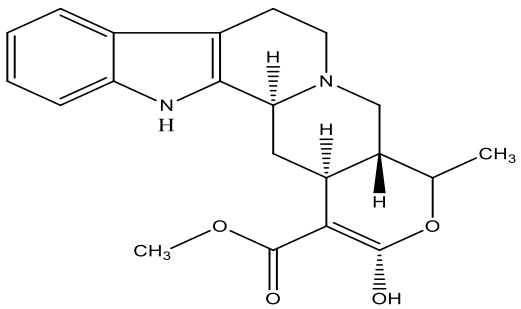


Figure 11. Structure of Ajmalicine.

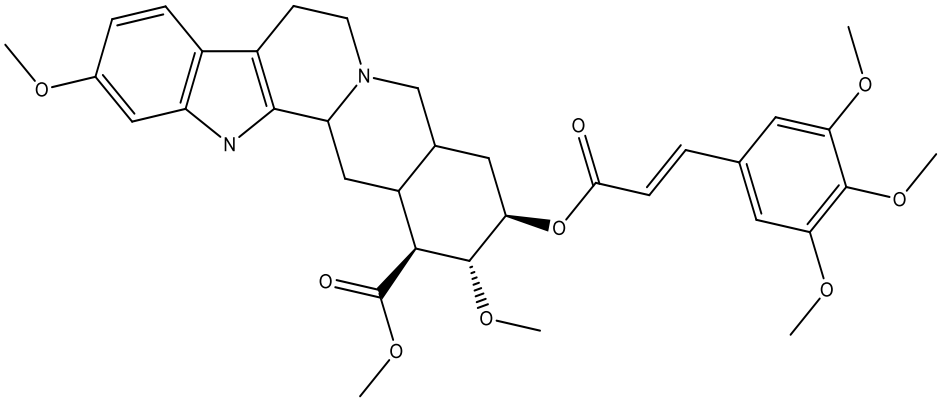


Figure 12. Structure of Rescinnamine.

***Marker compounds of* Rauwolfia serpentina**

Two major marker compounds of *Rauwolfia* sp. are Reserpine and Rescinnamine (Figure 12). In the plant reserpine concentration varied from 0.3% to 0.14% of the dry weight of the plant [54, 64, 65]. The resperine has higher content in roots and lower in the stems and leaves (Figure 13) [66].

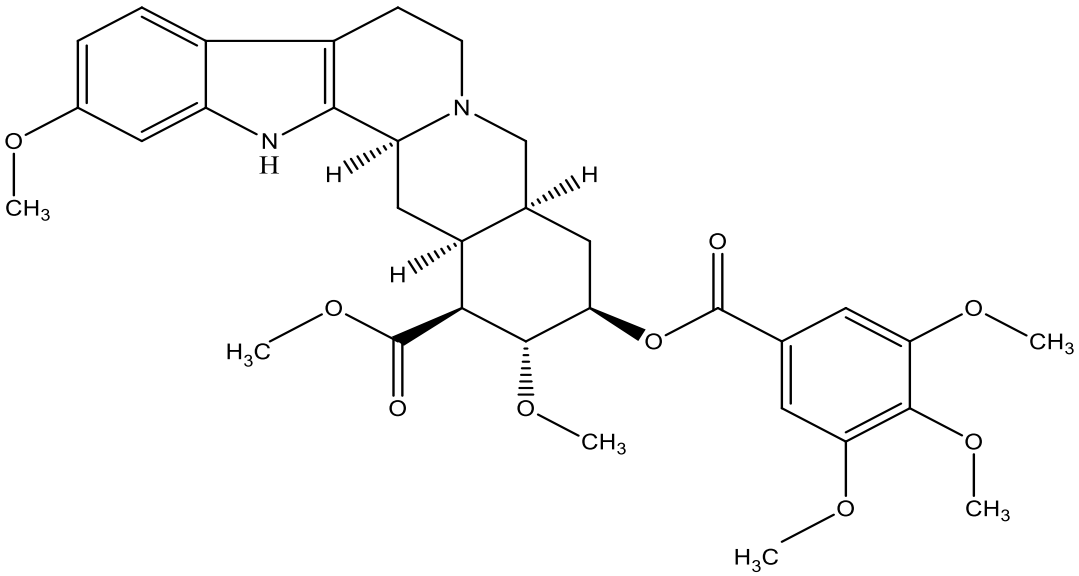


Figure 13. Structure of Reserpine.

***RAUWOLFIA* AND ITS ROLE IN MANAGEMENT OF HYPERTENSION**

In year 1949, an Indian homeopath treated fifty patients suffering from high blood pressure and hypertension using *Rauwolfia* tincture [67]. The author reported that 85% of patients showed decrease in systolic and 81% diastolic blood pressure. In year 1952, the reports were endorsed when in Germany and Austria, 25 patients treated by the *Rauwolfia* showed significant lowering in blood pressure. Meissner reported effectivity of *Rauwolfia* in 90% participants where the lowering of systolic blood pressure occurred in range of 15 and 40 mmHg [67]. The clinical use of a purified alkaloid mix called Alseroxylon was reported in the USA in 1952 [68]. Similarly, the reserpine and rescinnamine were mixture of the plant extract used for further studies. The one hundred patients were made to take *Serpina* product of *Rauwolfia,* the doses were given at the time interval ranging from a month to one year [69]. The serpina tablets were well tolerated for a daily dose showed in study, from 3 to 6 days action of serpina was moderated to appear as well as it disappeared 7 to 21 days after stopping the dose. It did not show any serious side effects, induced improved sleep and was not habit forming. A few patients however, reported drowsiness, nightmares, nasal congestion and bradycardia (slow heartbeat).

Rauwolfia serpentina was tested clinically for control of hypertension on 50 patients by Dr. Vakil, with initial blood pressure greater than 160/95 mmHg [67]. The study included 30 men, and 20 women aged 39 to 76 years. After one week of medication, 39 out of 48 patients showed a significant drop in low and high blood pressure [68]. The systolic blood pressure of patients further dropped between 2 and 54 mmHg after taking 4 weeks of medicines. In 22 of the 47 patients, 10 to 24 mmHg. Thirteen of the 47 patients had blood pressure below 25 mmHg. Also, in 38 subjects, diastolic pressure ranged from 4 to 34 mmHg. Diastolic blood pressure gradually decreased in 27 subjects, from 5 mmHg to 14. The hypotensive effect of the drug occurred within 15 days after discontinuation in 91% of patients and 30 days background in 75%.

Another clinical trial was conducted in an outpatient clinic to evaluate the effects of oral reserpine in a group of hypertensive patients. Reserpine, supplied by CIBA Pharmaceuticals, was administered to 15 subjects with baseline blood pressure ranging from 160/98 to 240/150 mmHg at a dose of 20 mg twice daily. For those patients, systolic circulatory strain dropped to a normal of 30.7 mmHg and diastolic pulse dropped a normal of 19 mmHg. A few patients reported nausea, blacking out and dyspnea. There researchers presumed that the medicine was a helpful and strong agent in certain patients with severe as mild hypertension. A review from EMBASE, and MEDLINE by Cochrane was embraced to research the portion related impacts of reserpine on heart rate, blood pressure, and withdrawals due to antagonistic effects [71].

The study chose really randomized, controlled preliminaries (RCTs) for audit that contrasted reserpine monotherapy with fake treatment or no treatment in patients with essential hypertension. Four RCTs were found to meet incorporation measures. None of the preliminaries detailed any withdrawals because of unfavorable impacts. The creators reasoned that reserpine was viable in decreasing systolic circulatory strain similarly as other preferred antihypertensive medications; however, they could not make clear ends in regard to the dose reaction design because of the modest number of included preliminaries. They proposed that more RCTs were expected to evaluate the impacts of reserpine on pulse and to decide the portion related wellbeing profile before the medication could be generally suggested as an essential antihypertensive medication. Reserpine is one of only a handful antihypertensive medications that have been displayed causing a decrease in RCTs mortality [72].

Rauwolfia serpentina and Management of Neurodegenerative Disease (NDD)

In past, the principal technique used to control the complexities of Alzheimer's disease (AD) was the restraint of acetylcholine esterase (AChE). Numerous phytochemicals of plant origin have been utilized to treat NDD. For the effective management of AD, a few medicines have been suggested by the physicians namely, rivastigmine, galantamine, and donepezil till 2014, except NMDA antagonist memantine [73]. In treatment of Parkinson’s disease, glaucoma, dementia, myasthenia gravis, AChE is a known procedure. For the AChE activity, various plant species have been screened [74]. Formation of receptive oxygen species, responsive nitrogen species is another significant neurotoxic pathway in AD, which prompts neuronal injury and death [75]. It led to an investigation where *R. serpentina* was utilized to screen against cholinesterase and cancer prevention agent activity [76]. The outcomes uncovered that R. serpentina showed high cancer prevention activity IC50 of 96 ± 7.8 µg/ml utilizing DPPH assay [77]. IC50 for AChE inhibitor was 22 ± 4.9 µg/ml utilizing Ellman’s colorimetric methodology [74]. Thus, R. serpentina was a compelling applicant as a wellspring of cell reinforcements and AChE inhibitors [78]. In another study by Saharia et al., reserpine viability in the cure of AD was evaluated. It showed that AChE was responsible for expanding the reserpine intervened lifespan [80] and decrease in Aβ (Amlyoid beta) toxicituy [79].

Reserpine: Mechanism of Action

The component of activity of reserpine has been reported in the past. Reserpine binds protein receptors called vesicular monoamine carriers (VMATs) bound with organelle layers of specific secretory vesicles at presynaptic nerve cells. Reserpine keeps intracellular synapses from restricting to

VMAT and prevents secretory vesicles from taking-up synapses. Eventually, utilization of reserpine gives that no or barely any synapses are delivered from the presynaptic neuron. Therefore, no or just slight proclamation of the nerve impulse happens in the postsynaptic neuron. VMAT1 and VMAT2 are two isoforms of vesicular vehicle proteins wherein VMAT1 has been initially found in the neuroendocrine cells of the PNS, especially in the chromaffin granules in the adrenal medulla, neurons, and platelets. VMAT2 is found in the brain and nervous system, while mast cells and histamine-producing cells are found in the gut and intestine. Reserpine has a partiality for VMAT2 that is multiple times more prominent than its liking for VMAT1. It has a solid affinity way, like it binds irreversibly to receptors on both VMAT and VMAT2 [81, 82].

MEDICINAL USES OF *RAUWOLFIA SERPENTINA*

In addition to the bioactive properties listed above, Rauvolfia has been studied for its ability to treat mood disorders such as schizophrenia and bipolar disorder, as well as seizures, epilepsy, insomnia, sleep disorders and anxiety [83, 84]. When the extracts from whole plant body were used in a study, including alseroxylon, reserpine, and the whole root, in the control of anxiety in ambulatory patients, similar results were obtained.

Rauwolfia has been studied in treatment for autism in mentally and physically retarded children of age group of 3.5 and 9 years [85]. Another study found that Rauwolfia helped prevent delirium tremens, a withdrawal symptom in patients addicted to alcohol and drugs. The researchers found significant reductions in violence, aggression, and severe depression. Additionally, Rauwolfia has been shown to effectively treat migraine headaches, improve quality of life, and reduce discomfort. It also shows promise in the treatment of patients with heart failure. It lowered the symptoms of angina and showed a prolonged therapeutic effect [88]. One-half of the patients in that study went on to develop normal ECGs. In dermatology, *Rauwolfia* has been found to be beneficial in treatment of pruritic, psoriatic and psychogenic dermatosis [89].

Rauwolfia and Control of Breast Cancer

The use of *Rauwolfia* and its alkaloids (reserpine) was pointedly decreased in the last part of the 1960s and mid 1970s when a supposed relationship to bosom malignancy was brought up in the clinical writing in 3 case-controlled studies [90]. A reassessment of the first investigations showed that those ends were incorrect. Avoidance inclination had happened on the grounds that patients with cardiovascular sickness were dismissed as potential controls in the comparison. Subsequent exploration and investigation wiping out avoidance inclination showed that no disease happened in those patients utilizing *Rauwolfia* or reserpine products [91]. The examination contrasted 257 ladies and bosom malignancy with 257 ladies that had no bosom malignant growth who were coordinated for age, date, affirmation determination, and race. The chances proportion of creating bosom malignancy was 1:1 when contrasting the individuals who utilized *Rauwolfia* items and the individuals who did not. At the point when 101 ladies with cardiovascular infection were rejected from the benchmark group in another investigation, the chances proportion rose to 1:2.5. The researchers reasoned that the outcomes proposed that rejection inclination assumed a significant part in making the bogus relationship among reserpine and bosom cancer.

Rauwolfia and Gastrointestinal Disorders

Rauwolfia extract of the roots used for treating intestinal disorders like stomach pain, liver pain and some other gastrointestinal disorder [93, 94].

Prostate Cancer

Prostate disease has been a significant cause of malignant growth amongst men. Current treatments for prostate cancer, such as chemotherapy and radiotherapy, offer little survival benefit [95]. Natural items have been demonstrated to be a significant asset for recognizable proof of bioactive mixtures utilized in the therapy of an assortment of illnesses and infections, including disease when contrasted with chemotherapy and radiotherapy. Different pieces of this plant have been consumed as a customary

medication for quite a long time to treat an assortment of infirmities including fever, general shortcoming, gastrointestinal sicknesses, liver issues and mental disorders [96]. The extracts from the roots of these plants are enriched with a combination of ß-carboline alkaloids, chiefly alstonine. This compound has been recently answered to diminish cancer cell development in mice vaccinated with YC8 lymphoma cells or Ehrlich austere cells (EAC). The plant removes has APC (Adenomatous Polyposis Coli) movement in both in vitro and in vivo model frameworks which, considering investigations of gene articulation examples of treated prostate malignancy cells, might be balanced by its consequences for DNA harm and cell cycle control [97].

- *In insanity*: *Rauwolfia serpentina* is accustomed to treating insanity. One gram of root powered can be taken two times in a day goat milk [98].
- *In itching skin:* One gram of root concentrate of *Rauwolfia* can be taken with water to alleviate itching in urtricaria [99].
- *Ethnoveterinary treatment:* The mixture of *Rauwolfia serpentina* roots 20 grams to 30 grams and 50 grams to 60 grams of sugar used to cure the loose motion in liverstock [99]. It is also used for menstrual disorder [100].
- *Spleen diseases: Rauwolfia* plants extract used for treating the spleen disease [82].
- *Eye treatment*: Fresh leaf juice of plant *Rauwolfia* used to prevent eye inflammation [101].
- *Treatment of Hysteria: Rauwolfia* is valuable in treating "Hysteria". 1 gram of root powder can be taken orally with milk three times in a day [98].
- *In sleeping disorder: Rauwolfia* is a small, realized cure for insomnia due to its properties. Doses ranging from 0.6 to 1.25 g of powdered intravenous extract are administered. 0.25 grams to the patient at sleep time for sound rest [98].

Miscellaneous Medicinal Properties of *R. serpentina*

Hypoglycemic: For checking the hypoglycemic activity in alloxan-injected diabetic rats methanolic extract of *Rauwolfia serpentina* was used and extract exhibited hypoglycemic, hypolipidemic activities and hepato-protective activities in alloxaninduced diabetics rats [53]. During *in vitro* studies, the extract of this plant showed coronary risk as well as antiatherogenic, glycemic the cardio protective in dice in alloxan induced diabetic mice [52].

- *Anxioytic:* Clinical study suggest that *Rauwolfia* can be used as anxiolytic agent, reserpine and alseroxylon or crude root exhibited anxiolytic action in ambulatory patients.
- *Anti-diarrheal:* An in vivo study in rats, where castor oil was deliberately used to induce seizures to investigate its anti-seizure effect, found that methanolic extracts *R. serpentina* is associated with anti-diarrhoeal activity and helped in reducing intestinal weight and fluid volume [53].
- *Antiarrhythmic:* In vivo studies in mice and cats showed that the anti-aerobic qualities of plant biomass grown in vitro released ajmalin derived from *Rauvolfia serpentina* Ajmalin, an anti-aerobic compound. mu-correction group I derived from the root tissue of the plant, also in the diagnosis of the congenital heart defect Brugada syndrome, it was useful.

CULTIVATION OF *RAUWOLFIA SERPENTINA*

The plant can be cultivated at anyplace in the fields, in evergreen timberlands and sub-Himalayan plots. The plant propagates best by root cuttings, seeds, and stem cuttings.

Land Preparation

The properly levelled field are the prime requirement for the cultivation of *R. serpentina*, rich in organic content, well drained, marginally acidic to marginally alkaline which pH going from 6.5 to 8 are reasonable for growth and, *Rauwolfia* is spread by two significant strategies (I) Seed (II) Cutting (Stem and Root)

Propagation and Plantation

Seed

Seed germination in *Rauwolfia* is an exceptional factor. Around 4–5 kg of new seeds are needed to cultivate for one hectare region and the appropriate time for nursery rising is of May to June. The

nursery is ready by raised beds according to necessity of planting material and simple for water system weeding and other intercultural activity with the FYM (Farmyard Manure). Around 500 sq m. space of seed bed is abundant for delivering seedlings enough for transplanting on one hectare of field. The seeds placed 3–5 cm separated in rows in shallow wrinkles. Seedlings are prepared after 40–50 days for transplanting in the field. The plant is transplanted at 30 x 45 cm distance inside line to line and plant to plant have been researched the culture strategies and propagation of *Rauwolfia* species by seeds [106–107].

Stem-Root Cutting

Badhwar et al. (1956) were accounted for the techniques for proliferation and their impact on root production in *R. serpentine* [108]. The stem cuttings of about 15 to 22 cm long stem cutting and 5 to 7 cm long root cutting are planted during June and July month in good to go nursery beds under control condition where moisture is maintained for a month. A normal 100–120 kg of new stem cuttings are discovered enough for planting in one hectare land. The cuttings start to grow inside 3 a month and after the growing of buds, branches and rising roots by the plants, these plants are prepared for transplanting at suggested spacing (Table 5).

Table 5. Ethnobotanical uses of *Rauwolfia serpentina* in India.

S.N.	Part Used	Disease Category	Mode of Use	State	References
1.	Root	Scabies	Mixed the fruits and roots of *R. serpentina* with lemon and boric acid used on infected parts.	Karnataka	(Parinitha et al., 2004) [102]
2.	Root paste	Itches and Eczema	Paste from root of *R. serpentina* used to cure disease	Orissa	(Behera et al., 2006) [103]
3.	Roots, seeds, fruits	Dog bite	Root extract used for treated the dog bites in 7days	South India	(Rahamatullah et al., 2010) [104]
4.	Root powder	Mental disorder	Powder root taken with honey or milk in morning to cure disorder	Orissa	Behara et al., 2006) [103]
5.	Leaves	Stomach pain	Powder mix with black pepper to relieve from stomach pain	Mayurbhani (orissa)	Rout et al., 2009) [105]

Manures and Fertilizers

The therapeutic plants typically no requirements of manures, pesticide and different synthetics so they effortlessly developed without utilizing of substance composts since they have own of insecticide, fungicide properties. The well decomposed FYM 10–15 tanks are mixed in soil before 15 days of plantation. Utilization of a wide range of natural fertilizers like FYM, Vermicompost, poultry excrement, and Green Manure improve the vegetative growth.

Irrigation and Intercultural Operations

For a good harvest of *Rauwolfia*, careful irrigation system should be essential. Plants require moisture when temperature increases and precipitation is deficient in rainy season. Normally, 15–20 water cycles are given during the whole yield time. If plants are grown in regions where annual rainfall of 150 cm or above is very much dispersed all throughout the growing season, it is recommended to irrigate crops fortnightly during hot dry season and once a month in winter.

Harvesting and Yield

At the point when plant transplanting is done in kharif or rainy season, the evacuating of roots is demonstrated with the yellowish color and shedding of plant leaves during the following year. At this stage, the roots find greatest solidification of all out alkaloids and root might be soil-deep up to 40–50 cm. The rootbark should not be harmed during harvesting since the bark contains a higher alkaloid rate contrasted with wood. After digging, the roots are cleaned and completely dried under shade condition to protect it from mould. The yields at 18 months term crop produce most extreme root yield. The 1.5 to 2 tans/ha of dry roots are acquired under the irrigated condition.

IN-VITRO MICROPROGATION OF *RAUWOLFIA SERPENTINA*

To meet the chemical demand of *Rauwolfia serpentina*, plant propagation is necessary for commercial cultivation. The roots of *Rauwolfia serpentina* mainly produce alkali. *Rauwolfia serpentina* roots are obtained through shoot organogenesis [109] or by callus morphogenesis (Table 6) [110]. Biotech approaches for *in-vitro* generation *R. serpentina* in laboratory by Ahmad et al. (2020) [111], and Pandey et al. (2010) [112].

In- vitro application of salinity stress in callus of *Rauwolfia serpentina*

Due to protein pattern changes, plant responses to salinity stress as well as biological changes occur in the adaptation process which help the plant become more fit to the altered environment [113] . In the *Rauwolfia* salinity stress leads to enhanced production of phenolic compounds. It has been reported salinity increases when polyphenolic content increases in different tissues. In the plant *R. serpentina* the total phenolic content increased with moderate salt concentration [114]. The antioxidant activity of Rauvolfia plants is due to the formation of various phenolic compounds and alkaloids (Table 7).

Singh and Patni used NaCl at different concentrations (10ppm, 15ppm, 20ppm,) in MS medium to observe the effect of saline soil on callus induction. Growth regulators 2,4-d, BAP were used in the same MS medium Figure 14(a-e).

Table 6. Effect of different growth regulators on formation of shoot and root in presence of MS solid media [111, 112].

	Shoots				**Roots**			
S. N.	***Growth regulators***	***Regeneration Response (%)***	***No of Shoots Per Culture***	***Shoot Length (cm)***	***Growth Regulators***	***Regeneration Response (%)***	***No. of Shoots Per culture***	***Root Length (cm)***
1	BA (0.5)	40.00	2.6 ± 0.27	3.5 ± 0.43	NAA (4.5)	52.23 ± 0.39^{a}	7.35 ± 0.72^{b}	5.22 ± 0.81^{ab}
2	BA (1.0)	46.66	3.3 ± 0.15	3.9 ± 0.20	NAA (5.5)	45.51 ± 0.52^{bc}	13.53 ± 0.65^{bc}	4.90 ± 0.31^{d}
3	BA (2.0)	60.0	3.9 ± 0.012	3.9 ± 0.18	NAA (6.0)	77.30 ± 0.87^{c}	12.37 ± 0.53^{de}	5.37 ± 0.42^{bc}
4	BA (2.5)	73.33	4.4 ± 0.39	4.2 ± 0.26	NAA (6.5)	52.76 ± 0.35^{bc}	7.51 ± 0.81^{c}	4.92 ± 0.34^{c}
5	BA (3.0)	46.66	3.2 ± 0.41	3.3 ± 0.39	NAA (8.5)	48.28 ± 0.44^{ab}	*8.93 ± 0.70^{cd}	5.95 ± 0.29^{a}
6	BA (3.5)	33.33	2.1 ± 0.30	3.0 ± 0.32	NAA (9.0)	89.52 ± 0.37^{c}	14.21 ± 0.66^{bc}	4.31 ± 0.78^{cd}
7	KIN (0.5)	33.33	2.0 ± 0.22	1.8 ± 0.52	NAA (9.5)	82.44 ± 0.53^{b}	*11.37 ± 0.43^{a}	6.18 ± 0.53^{ab}
8	KIN (1.0)	40.00	2.6 ± 0.31	2.9 ± 0.33	NAA (10.0)	52.80 ± 0.21^{de}	8.20 ± 0.75^{bc}	5.90 ± 0.28^{de}
9	KIN (2.0)	46.66	3.0±0.40	2.0 ± 0.18	PABA (0.5) + NAA (3)	31.55 ± 0.81^{c}	*5.72 ± 0.63^{ab}	5.55 ± 0.55^{a}
10	KIN (2.5)	60.00	3.8 ± 0.24	2.8 ± 0.27	PABA (1) + NAA (3)	71.17 ± 0.66^{c}	12.08 ± 0.32e	6.23 ± 0.35^{c}
11	KIN (3.0)	40.00	2.9 ± 0.15	2.6 ± 0.22	PABA (3) + NAA (3)	43.72 ± 0.25^{b}	*8.31 ± 0.85^{c}	5.76 ± 0.75^{bc}
12	KIN (3.5)	26.66	2.1 ± 0.21	2.0 ± 0.31	PABA (1) + NAA (4)	97.33 ± 0.45^{c}	37.10 ± 0.93^{d}	6.51 ± 0.87^{de}
13	BA + NAA (2.0 + 0.1)	80.00	4.3 ± 0.42	3.2 ± 0.51	PABA (2) + NAA (4)	58.69 ± 0.55^{a}	19.37 ± 0.59^{ab}	5.38 ± 0.64^{b}
14	BA + NAA (2.0 + 0.2)	40.00	2.2 ± 0.17	2.0 ± 0.41	PABA (3) + NAA (4)	92.40 ± 0.29^{bc}	*1.93 ± 0.38^{de}	4.55 ± 0.21^{cd}
15	BA + NAA (2.0 + 0.5)	-	-	-	IBA (3) + NAA (2)	63.94 ± 0.06^{e}	*7.39 ± 0.41^{bc}	4.89 ± 0.51^{a}

16	BA + NAA (2.5 + 0.1)	93.33	5.9 ± 0.63	3.9 ± 0.71	IBA (2) + NAA (4)	87.31 ± 0.37^{d}	23.85 ± 0.71^{a}	6.26 ± 0.75^{bc}
17	BA + NAA (2.5 + 0.2)	53.33	3.4 ± 0.59	2.6 ± 0.37	IBA (2) + NAA (4.5)	93.15 ± 0.19^{ab}	28.07 ± 0.33^{c}	6.33 ± 0.92^{d}
18	BA + NAA (2.5 + 0.5)	-	-	-	IBA (2) + NAA (5)	95.67 ± 0.33^{e}	30.51 ± 0.64^{bc}	5.85 ± 0.78^{c}
19	BA + IBA (2.0 + 0.1)	73.33	3.6 ± 0.64	2.7 ± 0.57	IAA (0.5) + NAA (2)	37.12 ± 0.59^{c}	2.91 ± 0.70^{d}	5.33 ± 0.19^{ab}
20	BA + IBA (2.0 + 0.2)	26.66	2.0 ± 0.29	2.0 ± 0.42	IAA (5) + NAA (5)	25.88 ± 0.08^{bc}	2.10 ± 0.43^{ab}	4.90 ± 0.37^{d}
21	BA + IBA (2.0 + 0.5)	-	-	-	BAP (5) + NAA (2)	97.30 ± 0.51^{ab}	*1.81 ± 0.51^{e}	3.24 ± 0.22^{c}
22	BA + IBA (2.5 + 0.1)	86.66	4.6 ± 0.32	3.1 ± 0.44	BAP (5) + NAA (3)	92.53 ± 0.73^{d}	*3.17 ± 0.43^{cd}	2.58 ± 0.57^{bc}
23	BA + IBA (2.5 + 0.2)	33.33	2.9 ± 0.43	2.1 ± 0.47	BAP (5) + NAA (3.5)	95.17 ± 0.44^{b}	*3.40 ± 0.82^{c}	2.73 ± 0.18^{e}
24	BA + IBA (2.5 + 0.5)	-	-	-				
25	KIN + NAA (2.0 + 0.1)	73.33	3.9 ± 0.32	2.8 ± 0.73				
26	KIN + NAA (2.0 + 0.2)	40.00	2.0 ± 0.23	2.0 ± 0.32				
27	KIN + NAA (2.0 + 0.5)	-	-	-				
28	KIN + NAA (2.5 + 0.1)	86.66	4.6 ± 0.71	3.2 ± 0.72				
29	KIN + NAA (2.5 + 0.2)	46.66	3.0 ± 0.29	2.1 ± 0.17				
30	KIN + NAA (2.5 + 0.5)	-	-	-				
31	KIN + IBA (2.0 + 0.1)	66.66	3.2 ± 0.55	2.3 ± 0.69				
32	KIN + IBA (2.0 + 0.2)	20.00	1.5 ± 0.61	1.0 ± 0.25				
33	KIN + IBA (2.0 + 0.5)	-	-	-				
34	KIN + IBA (2.5 + 0.1)	80.00	4.1 ± 0.83	2.6 ± 0.42				
35	KIN + IBA (2.5 + 0.2)	26.00	2.6 ± 0.22	1.8 ± 0.44				
36	KIN + IBA (2.5 + 0.5)	-	-	-				

*NAA: Naphthalene acetic acid; PABA: para-amino benzoic acid; IBA: Indole 3-butyric acid; BAP: Benzyl aminopurine. BAP: 6-benzyl aminopurine; IAA: Indole-3-acetic acid; BA: 6-benzyl adenine; KIN: kinetin * callus growth.*

Table 7. Effect of saline conditions on phenolic content, superoxides dimutase and PAL in callus of *Rauwolfia serpentina* at different interval of days [115].

Chemical Used for Salinity Stress Conc	**Phenolic Acid Content (mg GA g-1 FW) at Different NaCl conc. (in Number of Days)**			**Superoxide Dimutase Activity (units/mg protein) at Different NaCl Concentration (in Days)**			**Phenylalanine Ammonia-lyase Activity at Different NaCl conc**		
	5	10	15	5	10	15	5	10	15
Control	1.2	3.21	3.5	6	8.33	10.27	1.2	1.35	1.9
10 ppm	2.3	3.7	4.09	7.22	11.66	12.33	1.5	2.07	2.28
15 ppm	3.97	4.34	5.20	8	14	16.9	1.64	3.14	3.99
20 ppm	2.5	3.80	4.5	8.4	16.66	20	2.4	4.5	5.4

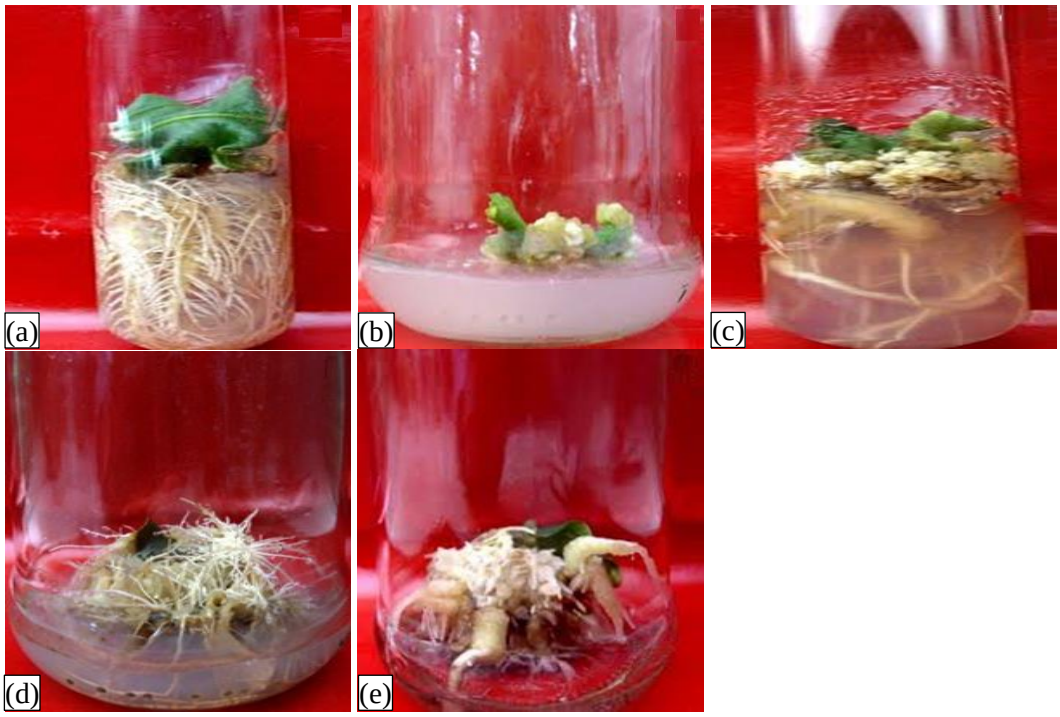


Figure 14. Response of leaf explants of *Rauwolfia serpentina* on MS media. Where, A = root induction on MS media containing 1 mg l-1 PABA + 4 mg l-1 NAA; B = callus initiation on MS media containing 0.5 mg l-1 BAP + 2 mg l-1 NAA; C = callus and root induction on MS media containing 5 mg l-1 BAP + 3 mg l-1 NAA; D = root induction on MS media containing 1mg l-1 PABA + 4 mg l-1 NAA under dark conditions; E = root induction on liquid MS media containing 1 mg l-1 PABA + 4 mg l-1 NAA (Pandey *et al*., 2010) [115].

1. *Test for saponins (forth test):* To 5 ml of distilled water taken in test tube, added a pinch of the dried powdered plant material and the mixture was vortexed. The foam formation shows that the sample is positive for saponin.
2. *Test for triterpenoids (Salkowski's test):* To the test solution, added drops of concentrated H_2SO_4. The mixture was vortexed and allowed to stand for some time. Two colors appeared: red at the lowest level, indicating the presence of sterols; and red, indicating the presence of triterpenoids.
3. *Test for flavonoids: Sulphuric acid (H_2SO_4 test):* The test solution was treated with concentrated H_2SO_4. Orange color indicates the presence of flavonoids in the sample.
4. *Test for phenolic compounds (ferric chloride test):* A small amount of ferric chloride reagent was mixed with the test solution. A vivid green, red, blue, or black color indicates the presence of tannins.
5. *Tests for alkaloids:*
   i. *Dragendorff's test* – To 5 ml of the filtrate, added few drops of Dragendorff's reagent, formation of orange-brown precipitate shows presence of tannins.
   ii. *Mayer's test* – To 5 ml of the filtrate, added 2 drops of Mayer's reagent and observe for formation of precipitate.
   iii. *Hager's test* – To 5 ml of the filtrate, add a few drops of Hager's reagent. Observe the formation of yellow precipitate.
   iv. *Wagner's test* – Add a few drops of Wagner's reagent to 5 ml of filtrate. Observe formation of reddish-brown precipitate.

Qualitative and Quantitative Analysis of Indole Alkaloids of *R. serpentina*

One of the best methods for the alkaloid analysis both qualitative as well quantitative is HPLC because of its resolution power and automatization. Different stationary phases were used in the past for normal phase packing, example: Aluminum Oxide [117].

Qualitative Analysis

Thin Layer Chromatography

Qualitative assessment of the main family of indole alkaloid compounds from Rauwolfia started with thin layer chromatography (TLC) on preparative silica gel porosity-60. A 97:3 mixture of chloroform and methanol was used in the mobile phase though extracted alkaloids. Consistent images of the fibers resulted when plates were sprayed with Dragendorf reagent or examined under an ultraviolet transilluminator. They were identified using the color and Rf values of the fibers under UV light [118, 119].

High Performance Liquid Chromatography

The authors used Lichrosorb C HPLC to analyze alkaloid contents of crude aliquot of *R. serpentina* with Lichrosorb C–18 (25 x 0.5 cm $10A^0$) column and Acetonitrile: Phosphate Buffer (35:65) was used as mobile phase. A total of 20 ml sample was loaded at flow rate of 1mL min^{-1} using microliter syringe. The detection wavelength was kept at 268 nm and the processing was done at ambient temperature. The chromatogram of R metabolites was obtained using the isocratic method *serpentine* [120, 121].

Quantitative Analysis

Antibacterial activity

Antibacterial activity of crude *R. serpentina* extract was determined by agar well diffusion method [122]. Antibacterial activity of root and leaf extracts was examined against four bacterial samples like *Salmonella typhii, Styaphylococcus aureus, Eschrichia coli* and *Bacillus subtilis* for antibacterial potential of the extract. The results were compared with killing activity of two antibiotics namely streptomycin and ampicillin (Table 8) [122].

Table 8. Comparison of antibacterial potential of *Rauwolfia* extracts with antibiotics [122].

Test Organism	Diameter of Zone of Inhibition (mm)			
	Root Sample	***Leaf Sample***	***Streptomycin***	***Ampicillin***
E. coli	15.5±0.5	7.4	36	35
S. typhii	22.5±2.5	10.1	42	35
S. aureus	13.5±3.5	9.0	29	25
B. subtilis	17.5±0.5	9.5	37	25

The root extract of *Rauwolfia* showed the efficient restricting activity against *S. typhii* bacteria.

Minimal Inhibitory concentration–MIC

The noise dilution method was established by Alade Irabi in 1993. After testing for bactericidal activity, the extracts with positive results were selected for MIC determination by incubating bacterial cultures for 16 h and diluting them with crude salt do not use (0.85% sodium chloride). About 10^6 colony forming units can be inoculated We have, after several dilutions with water, the final concentration ranged from 1 to 100 mg per milliliter. Each tube was loaded with 20 µl of bacterial suspension per milliliter of nutrient solution and incubated overnight at 37° C. The MIC was calculated as the lowest concentration of the extract in the broth that inhibited the growth of the tested microorganisms. After 24 hours, the MIC was determined by measuring the optical density of each sample at 600 nm using a spectrophotometer and comparing with the results of the nutrient suspension [124].

Minimum Bactericidal Concentration

After determining the MIC of the alkaloids, a 10 µl aliquot of each tube with no evidence of bacterial growth was transferred to nutrient agar (NA) plates and these plates were then incubated at 37° C for 24 h. Bacteria a minimum bactericidal concentration (MBC) and a minimum bactericidal concentration that removes more than 99.9% of the initial bacterial population, while bacterial growth is evident on the NA plate.

Antiproliferative Activity

In the cervical cancer cell line (HeLa), *E. coli*. Aliquots of 250 µl of culture medium were added to sterile water, followed by 50 µl of extract solution at different concentrations (50, 100, 150, 200 µg/mL in ethanol) and then the vials into a CO2 incubator and incubated at 37°C for 48 hours. After consumption, the vials were centrifuged, and the supernatant was discarded. Each vial received 600 µl of fresh BSS medium and 30 µl of MTT working solution. The vials were then incubated for another 1–4 h. After incubation, add 450 µl of an aqueous solution such as dimethyl sulfoxide, acidified ethanol, or sodium dodecyl sulfate.

CHROMOSOME ANALYSIS

R. serpentina is classified as a general species based on its chromosome size, length, and centromere type. The CMA banding method was used to identify GC-rich chromosomal regions. This method also detected multiple cell types and polyploidities in *Rauwolfia*. In the year 1962, the presence of tetraploidy in *R. serpentina* was reported. Root yield of *Rauwolfia* increasing due to colchiploidy was reported by year 1965 and 1968 [125].

ORGANOLEPTIC AND MICROSCOPIC EVALUATION OF *RAUWOLFIA* ROOTS

Organoleptic evaluation of both wild and cultivated roots of *Rauwolfia* was done. Evaluation provides the identity and purity as well as ensuring the quality of a roots (Table 9) [125].

Microscopic evaluation of *Rauwolfia* roots

Microscopic and macroscopic evaluation of wild and cultivated roots of this done by standard methods (WHO 1996) [127].

Table 9. Analysis of *Rauwolfia serpentina* mother tincture from wild and cultivated roots [128].

S.N.	Test Parameter	Wild Roots	Cultivated Roots
1.	Mother tincture coloration	Clear dark brown solution	Clear dark brown solution
2.	Alcohol percentage	75% v/v	73% v/v
3.	pH	6.15	6.10
4.	Weight per ml	0.87 g ml^{-1}	0.88 g^{-1}
5.	Total solids	1.714	1.354

IN VIVO EFFECT OF METHANOLIC ROOT EXTRACT OF *RAUWOLFIA* SERPENTINA ON ALLOXIAN INDUCED DIABETIC MICE

During the last two decades, diabetes has emerged as one of the alarmingly rising disorders in populations of developed and developing nations [129]. Diabetes is a metabolic disease, characterized by hyperglycemia, due to reduced or no secretion of insulin. It has alone caused more than 5% of deaths annually worldwide [130, 131]. The commercially available allopathic pharmaceutical formulations used for curing diabetes have severe side effects, when used for longer durations. Further, these drugs do not restore the glucose homeostasis on permanent basis, a suggested dose has to be taken repeatedly by the patient to maintain blood glucose level (Figure 15). It has been reported that since the last decade, about 80% of world population has shifted/rely on herbal medicines (Table 10).

It has been reported herbal medicines have been significant contribution for the treatment of diabetes as well as endocrine disturbance in all over the world (Table 11). The authors have reported the activity of methanolic extract of *R. serpentina* has been reported in alloxan – induced diabetic rats [53]. Glucose tolerance activity in mice or quantitative and qualitative phytoconstituents analysis have been done by Azmi and Qureshi 2012. *R. serpentina* root bark is rich in alkaloids like ajmaline reserpine etc. Azmi *et., al*; 2013 consolidated their previous findings by extensively studying the effect of methanolic root extract on mice. The authors studied the effect on body weights, glycemic, antioxidant enzyme, glycosylation HbA1c, Hb ratio etc. (Table 12). The study concluded that all test parameters were limited to permissible level by giving various doses of MREt at different times.

(a) (b)

Figure 15. The Wild roots (yellow to brown; left side) and cultivated roots (greyish yellow to light brown; right side) of *R. serpentina.*

Table 10. Acute toxicity of MREt of *Rauwolfia serpentina* [51].

S.N.	Treatment	Hypoglycemic Activity*	Sedative Behavior	% Mortality Rate
1.	Control distilled water (1 ml/kg)	119.33 ± 12.56	–	0
2.	MREt of *R. serpentina* (10 mg kg^{-1})	80 ± 2.56***(–33)	–	0
3.	MREt of *R. serpentina* (30 mg kg^{-1})	65 ± 1.23***(–45)	–	0
4.	MREt of *R. serpentina* (60 mg kg^{-1})	62 ± 2.83***(–48)	–	0
5.	MREt of *R. serpentina* (100 mg kg^{-1})	55 ± 3.56***(–54)	+	17
6.	MREt of *R. serpentina* (150 mg kg^{-1})	26 ± 0.82***(–78)	++	50
7.	MREt of *R. serpentina* (200 mg kg^{-1})		+++	83
8.	MREt of *R. serpentina* (250 mg kg^{-1})		+++	100

Table 11. Effect of MREt of *R. serpentina* on blood glucose level in mice [51].

S.N.	Groups	Treatments	Initial Day (G)	Final Day (G)	Glycemic Change %
1.	Group I	Distilled water (1ml/kg)	102.25 ±2.72	98 ± 5.84	–4.16
2.	Group II	Alloxan treated (150mg/kg)	198.25±4.91	245.50 ± 20.66	23.83
3.	Group III	0.05%DMSO (1ml/kg)	199.5± 5.62	257.25 ± 22.68	28.95
4.	Group IV	Glibenclamide (5 mg/kg)	195.25 ±3.12	116.50 ± 14.46****	–40.46
5.	Group V	MREt (10mg/kg)	198.25± 5.01	96.25 ± 4.94****	–51.45
6.	Group VI	MREt (30mg/kg)	197.50 ± 6.2	105 ± 12.39****	–46.84
7.	Group VII	MREt (60mg/kg)	195.2 ± 5.66	98 ± 6.54****	–49.80

Table 12. Effect of MREt on complete blood profile [51].

S.N.	Group	R.B.C(10^6/uL)	W.B.C(10^3/uL	P.C.V(%)	M.C.V(fL)	M.C.H(Pg)	M.C.H.C(g/dL)
1.	I	4.85±0.29	3.5±0.47	22.93±2.64	46.20±2.91	17.22±1.09	34.78±2.24
2.	II	3.15±0.39	5.08±0.44	18.30±2.01	36.36±3.63	14.49±1.05	20.21±2.77
3.	III	3.45±0.35	5.40±0.44	17.93±2.80	36.15±3.24	13.46±1.30	22.76±2.27
4.	IV	3.6±0.36	3.08±0.30	23.28±2.98	45.84±1.57	17.94±1.35	35.41±2.25
5.	v	4.38±0.29*a	2.88±0.46**a***b	23.91±2.58	52.19±2.98***ab	16.84±1.88	32.15±1.84**a**b
6.	vi	4.68±0.33*ab	3.55±0.52*ab	28.1±3.55*ab	53.36±2.25***ab	18.02±1.98*b	34±2.68***a*b
7.	vii	5.68±0.33***a**b	2.88±0.30**a***b	29.51±4.31*ab	55.13±2.12***ab	19.50±1.39*ab	36.89±1.64***ab

CONCLUSION

Rauwolfia serpentina has proved to be a wonder drug. It has been proved as a boon to cure a variety of disorders and diseases of mankind. It has been extensively used in all the systems of medicines worldwide and it is an important herb with vast medicinal properties. In the past, it has proven its efficacy for management and treatment of highly complex human disease. The *in vivo* and *in vitro* case studies and related clinical data have shown it to be anti-hypertension, anti-diarrheal drug with minimum of side effect hence advocating further extensive studies involving *R. serpentina* in curing and management of human disease.

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