

**COMPARATIVE PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION  
OF CULTIVATED AND WILD VARIETY OF *RAUWOLFIA SERPENTINA*  
(APOCYNACEAE)**

A THESIS  
SUBMITTED IN PARTIAL FULFILLMENT  
OF THE REQUIREMENTS FOR THE DEGREE OF  
**MASTER'S OF PHARMACY (AYURVEDA)**  
IN  
**DRAVYAGUNA VIJANANA**  
(AYURVEDIC PLANT SCIENCE)

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*Transforming Education Transforming India*

**LOVELY SCHOOL OF AYURVEDIC PHARMACEUTICAL SCIENCES  
LOVELY FACULTY OF APPLIED MEDICAL SCIENCES  
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PHAGWARA, PUNJAB-144411  
MAY, 2017**

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This is to submit that this written submission in my dissertation report entitled “**Comparative Pharmacognostic & Phytochemical Evaluation of Cultivated and Wild variety of *Rauwolfia serpentina* (Apocynaceae)**”. It represents original ideas in my own words and where others’ ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I comprehend that any infringement of the above will be cause for disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly referred or from whom proper permission has not been taken when required. I guarantee and hold full obligation regarding its validity.

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The work described in this project report entitled “**Comparative Pharmacognostic & Phytochemical Evaluation of Cultivated and Wild variety of *Rauwolfia serpentina* (Apocynaceae)**” has been carried out by **Saveena Chauhan** under my supervision. I certify that this is his genuine work. The work described is original and has not been submitted for any degree to this or any other university.

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## LIST OF ABBREVIATIONS

<b>ESA</b>	Endangered Species Act
<b>NMPB</b>	National Medicinal Plants Board
<b>AYUSH</b>	Ayurveda, Yoga And Naturopathy, Unani, Siddha And Homoeopathy
<b>NAM</b>	National AYUSH Mission
<b>T.S.</b>	Transverse Section
<b>HPTLC</b>	High Performance Thin Layer Chromatography
<b>WHO</b>	World Health Organization
<b>GC-MS</b>	Gas Chromatography In Combination With Mass Spectroscopy
<b>mg</b>	Milligram
<b>gm</b>	Gram
<b>FRAP</b>	Ferric Reducing Ability Of Plasma Or Plants
<b>DPPH</b>	2,2-Diphenyl-1-Picrylhydrazyl
<b>LDL</b>	Low Density Lipids
<b>ACE</b>	Angiotensin-Converting-Enzyme
<b>LOD</b>	Loss On Drying
<b>TLC</b>	Thin Layer Chromatography
<b>ppt.</b>	Precipitates
<b>TPC</b>	Total Phenolic Content
<b>°C</b>	Degree Celsius
<b>µL</b>	Microliter
<b>MHA</b>	Muller Hinter Agar
<b>min.</b>	Minute
<b>s</b>	Second
<b>nm</b>	Nanometer
<b>hrs.</b>	Hours
<b>Std.</b>	Standard
<b>Rf</b>	Retention Factor

## ABSTRACT

Herbal plants are the essential components of traditional medicines in several countries. The plant *Rauwolfia serpentina* commonly known as Sarpagandha has been used in India from century for curing countless diseases, for example hypertension, mental disturbance, epilepsy, injuries, tension, energy, schizophrenia, sleeping disorder and insanity. The present work focuses on the comparative pharmacognostic, analytical and pharmacological studies between wild and cultivated variety of *Rauwolfia serpentina*. The wild variety of plant shows the more number of layers and difference in cells as compare to the cultivated one. The samples are evaluated for the identity, purity and strength which also show somewhat similarities in both plants except in the alcoholic extractive value which is more in case of cultivated plant. The phytochemical screening shows the presence of alkaloids, tannins, flavanoids, carbohydrates, glycosides in the both samples and few amount of steroids and proteins. Tannic acid content is found to be more in case of wild variety and phenolic content is approximately similar in both of the cases. TLC and HPTLC studies of the plants shows the presence of various constituents in it and in comparison with the standard Reserpine, the peak is found at Rf about 0.6 in both the samples. The microbial and - amylase inhibition is moreover similar but in case of antioxidant activity the wild source of the plant have more potential to exert antioxidant activity as compare to the cultivated variety. As we know the plant *Rauwolfia serpentina* is in the endangered category and found to be extinct so this research helping us to save the endangered plant by giving its substitute which is moreover similar to this in every aspect.

**Key words:** *Rauwolfia serpentina*, Sarpagandha, Reserpine, Antioxidant activity, Antidiabetic activity.

# CHAPTER I

## INTRODUCTION

### 1.1 Introduction to Ayurveda

Ayurvedic system of medicine has its roots since antiquity and it deals with drugs from herbal, mineral and animal origin. It encompasses all aspects of life. Ayurveda works on the objective of maintaining the health of a healthy person and curing the ailments of ailing by using drugs of different origin<sup>1</sup>.

स्वस्थस्य स्वास्थ्यरक्षणं आतुरस्य विकारप्रशमनं ॥

Various Ayurvedic or herbal drugs are reported in classical literature as a good source of medicine.

### 1.2 Introduction to Dravyaguna

Dravyaguna deals with the study of dravya (drug) used in ahara (diet) and ausadha (medicine).

द्रव्याणां गुणकर्माण प्रयोगाः विविधास्तथा ।

सवशो यत्र वण्यन्ते शास्त्रं द्रव्यगुणं हि तत् ॥

The first mentioning of dravyaguna is traceable from the descriptions of Charaka who defined Ayurveda as the science which deals with the dravya, gunas and karma which are helpful and harmful materials. Dravyaguna Sashtra has been identified as a separate speciality by Nirahari, the author of Raj Nighantu. Ayurveda advocates the concept of pharmacotherapeutics and clinical pharmacology but not pharmacology and therapeutic separately<sup>2</sup>.

### 1.3 Importance of herbal plants

Plants are imperfect men. The human progress of the Indian society is begun at wilderness. Our precursors gave more significance to the plants. It is well explained in classical text like Veda, Ramayana-Mahabharata etc. One of the synonym for plant is 'taru' means by which the men can be free from sadness<sup>3</sup>.

‘तरन्ति आपदं अनेन इति तरूः’

So the plants are the important part of our life since ancient time. Herbal medicines or aushadhi means: one which relieves the pain (vednasthapan) and which have potency.

Numbers of plant are reported to have medicinal property and one of the important plant is Sarpagandha. This perennial herb is *Rauwolfia serpentina* which belongs to family

Apocynaceae. It is widely distributed in moist area in subtropical Himalayas and plains and roots are mainly used for medicinal purpose<sup>4</sup>.

#### **1.4 Endangered species**

The plants and animal species that exist in the less number and are in danger of becoming extinct in future are endangered species. Endangered Species Act (ESA) was passed to protect those species in the year 1973. ESA classified into two categories, "Threatened" or "Endangered", depending on their status (left in wild area) and how severely their survival is threatened. Threatened species are those which are probably to become endangered in the future<sup>5</sup>. Example of some of endangered species are: *Mesua ferra* (Guttiferae), *Swertia chirayita* (Gentianaceae), *Alstonia scholaris* (Apocynaceae), *Acacia catechu* (Leguminosae), *Pterocarpus santilanus* (Leguminosae), *Butea monosperma* (Leguminosae), *Michelia champaca* (Mangoliaceae), etc. The plant *Rauwolfia serpentina* also comes under the category of endangered species. Over-extraction for market use, reduced restoration, expansion of agriculture, deforestation and arbitrary use of pesticides in modern agricultural practices to control weeds and insects are the major cause behind dwindling population of Indian snakeroot in India.

#### **1.5 Role of Government for herbal plants:**

Government of India has set up National Medicinal Plants Board (NMPB) under the department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) in 2000 to look after the matters that are related to medicinal plants. NMPB continuously work towards the promotion of medicinal plant cultivation, conservation and development. Presently they are implementing new schemes in order to develop medicinal plant sector:

1. Central Sector Scheme for Conservation, Development and Sustainable Management of Medicinal Plants.
2. Centrally Sponsored Scheme of National AYUSH Mission (NAM)<sup>6</sup>.

8,000 medicinal plants and 53 species are under threat as per Botanical Survey of India.

#### **1.6 Importance of soil/climatic conditions:**

Soil is the most essential characteristic asset for the growth of any plant. *Rauwolfia serpentina* grow under a wide range of temperature (10<sup>0</sup> to 30<sup>0</sup>), rainfall (2500-4000 mm), and soil (sandy alluvial loam to red lateritic loam with pH 4-8.5). Other necessary nutrients can be provided through manure, fertilizers etc<sup>7</sup>.

**CHAPTER II**  
**TERMINOLOGY**

<b>Dravyaguna vijnana</b>	<b>Dravyaguna vijnana</b> is that branch of science which deals with dravya (drug and diet).
<b>Dravya</b>	<b>Dravya</b> is substance or which act as abode for guna ( properties) and karma (actions)
<b>Samhita</b>	<b>Samhitas</b> are the classical text which are taken as reference
<b>Nighantu</b>	<b>Nighantus</b> are the vocabulary which includes description of plants and other definitions
<b>Rasa</b>	<b>Rasa</b> is the taste of any of the substance or the object of gustatory sense organ which is located in the tongue
<b>Guna</b>	<b>Guna</b> are the properties of any substance
<b>Virya</b>	Effectiveness and potency of all the guna is called as <b>virya</b>
<b>Vipaka</b>	Transformed state of the ingested substance after digestion is called as <b>vipaka</b>
<b>Prabhava</b>	The specific potency of the drug is called as <b>prabhava</b>
<b>Standardization</b>	It is the process to ensure the identity , quality, purity and efficacy of crude drugs by means of various parameters
<b>Wild source</b>	The herbs which are collected from their natural or indigenous source are known as <b>wild source</b> of plant
<b>Cultivated source</b>	The herbs which are grown by cultivation for mass production are known as <b>cultivated source</b> of plant



## CHAPTER III

### REVIEW AND LITERATURE

A literature review is a text of a learned paper, which comprises the current knowledge including practical findings, as well as academic and procedural contributions to a particular topic. It helps to identify the gaps of studies of particular thing and also recommend new area of exploration.

#### 3.1 Significance of Review of Literature:

- Provide the theoretical background of the study.
- Appropriate raw drug can be selected with the help of their classical synonyms.
- All progress is born of inquiry.
- Various therapeutic effects can be studied further.
- Provides up-to-date understanding of subject and its significance to practice.

#### 3.2 Ancient Literature

The first reference of the *Rauwolfia serpentina* is found in the pre-vedic period. There is much folklore for this plant. In Bihar it is renowned as “pagal ki jaddi-buti” because of its curative effect in case of insanity<sup>3</sup>. Mongoose would like to chew its leaves before fighting with cobra for strengthen his body.

##### 3.2.1 Charaka Samhita

वचां वंशत्वचं पाठां नतं सुरसमञ्जरोम्।

द्वे बले नकुला कुष्ठं शिरोषं रजनीद्वयम्॥

The plant nakuli is kept in param agad (vachadi yoga) and it is one of the synonym of Sarpagandha<sup>8</sup>.

##### 3.2.2 Sushruta Samhita

कुक्कुटा सवगन्धा च तथा कार्णावकार्णिके ।

वज्र प्रोक्ता वयःस्था च शृङ्गी मोहनवल्लिका ॥

अकमूलं त्रिकटुकं लता स्त्रोतोजमञ्जनम् ।

नैपालो हरितालञ्च रक्षोघ्ना ये च कार्तिताः ॥

The classical text, Sarpagandha is included in Aparajit Gana which is indicated in mental disorder (Susruta Uttartantra 60/47). Sarpandha is also included in Ekasar Gana (Susruta Kalpa 5/84) useful against visha and for treatment of Musaka Visha (Susruta Kalpa 7/29)<sup>9,10</sup>.

### 3.2.3 Vrindhamadhava

Also use in treatment of Visuchika. (Vrindamadhava 6/26)<sup>10</sup>.

## 3.3 Medieval Literature

### 3.3.1 Dhanvatari Nighantu

नाकुलो सपगन्धा च सुगन्धा भोगिगन्धिका ।

सैव सपसुगन्धा च तथा चीरितपत्रिका ॥

नाकुलो कटुरूष्णा स्यात्तिक्ताऽपि परिकीर्तता ।

मूषिकस्य विषं हन्ति कृमिदोषविनाशिनी ॥

It is explained as the name of sarpagandha and various other names as Nakuli, Katuushna, Vishhanti etc<sup>11</sup>.

अन्या महासुगन्धा च सुवहा गंधनाकुलो ।

सपाक्षी नकुलेष्टा च च्छत्राको विषमदिनी ॥

### 3.3.2 Jaimini brahman

नाकुलिभस्त्रासप्रियंगुतण्डुलानां वा पूणा ।

Jaimini brahman also explains about Sarpagandha as Nakuli<sup>2</sup>.

## 3.4 Modern literature

### 3.4.1 Bhavaprakash

नाकुलो सुरसा नागसुगन्धा गन्धनाकुलो । नकुलेष्टा भुजङ्गाक्षी सपाङ्गी विषनाशिनी ॥

नाकुलो तुवरा तिक्ता कटुकोष्णा विनाशयेत् । भोगिलूतावृश्चिकाखुविषज्वरकृमिघ्नान् ॥

In Bhavaprakash Nighantu Sarpagandha is taken as type of Rasna and explains as the name of Nakuli, Sursa, Sugandha, Gandhanakuli, Nakuleshtha, Sarpangi, Dhawalbarua, Vishnashini, Tikta, Katushna<sup>12</sup>.

### 3.4.2 Shodhal

नाकुल्यां गंधमूला च सुगंधा सुवहा च सा ।

सुरभी सपगंधा च गंधाख्या गंधचारिणी ॥

Acharya Shodhal explained the plant as Gandhamula, Sugandha, Vatavyadhinashini.

### 3.4.3 Kaidev Nighantu

In this text the drug is explained as Nakuleshtha, Mahaveerya, Kashayoushna.

### 3.4.4 Raj Nighantu

नाकुलो सपगन्धा च सुगन्धा रक्तपत्रिका ।

इश्वरो नागगन्धा चाप्याहभुक् स्वरसा तथा ।

सपादनी व्यालगन्धा ज्ञेया चेति दशाहव्या ॥

Nakuli sarpagandha, sugandha, raktapatrika, ishwari, naggandha, ahibhuka, svarasa, sarpadini, and vyalgandha these are the ten names of Nakuli<sup>13</sup>. Explained as the name Sarpakshi, Sarpatani Tikta and Katu rasa and Tridoshajeeta.

### 3.4.5 Priyangu Nighantu

ईषन्नीलारूणसुमदला पुष्पिता ग्रीष्मकाले,

वषाकाले फलपरिचितं नीलरक्तां दधाति ।

मूलं यस्या हरिणकर्पशं स्थूलमन्तःस्थचक्रं ,

चन्द्राख्या सा धवर्लावपटा सपगन्धा प्रसिद्धा ॥

Also explains about Sarpagandha plant and its various synonyms, uses etc<sup>14</sup>.

### 3.4.6 Description of plant *Rauwolfia serpentina*

Drug consists of dried roots of *Rauwolfia serpentina* (linn.) Benth. ex Kurz (syn. *Ophioxylon serpentinum* Linn.); Family: Apocynaceae<sup>4</sup>.

### 3.4.6.1 Various synonyms of the plants are reported in table no. 3.1

**Table 3.1: Synonyms of *Rauwolfia serpentina***

S. No.	Language	Names
1.	Sanskrit	Nakuli <sup>8,2,11,12,13,15,17,19</sup> , Sarpagandha <sup>9,2,11,13,3,16,18,19,20</sup> , Sugandha <sup>11,2,13</sup> , Bhogigandhika <sup>11</sup> , Sarpasugandha <sup>11,2</sup> , Cheeritpatrika <sup>11</sup> , Mukta <sup>2</sup> Vishmardini <sup>11</sup> , Mahasugandha <sup>11</sup> , Chhtraki <sup>11</sup> , Suvaha <sup>11</sup> , Sarpakshi <sup>11,19</sup> , Nakuleshtah <sup>11,2</sup> Sursa <sup>12</sup> , Nagasugandha <sup>12</sup> , Bhujangi <sup>12</sup> , Sarpaangi <sup>12,19</sup> , Vishnashini <sup>12</sup> , Ishwari <sup>13</sup> , Raktapatrika <sup>13</sup> Ahibhuka <sup>13</sup> , Swarasa <sup>13</sup> , Sarpadini <sup>13</sup> , Naganadha <sup>13</sup> , Vyalgandha <sup>13</sup> , Dhavalvipata <sup>20</sup> , Chandrika <sup>14,16,17,18</sup> , Gandhanakuli <sup>15</sup> , Chandramarah <sup>17,20</sup> , Dhavalavitapa <sup>14,19</sup>
2.	Hindi	Dhavalabaruaa <sup>4,17,20</sup> , Chandmarvaa <sup>4,11,12</sup> , Chota chand <sup>12,16,18</sup> , Nakulkanda <sup>12</sup> , Nakulikanda <sup>3</sup> , Naii <sup>3</sup> , Harkaii chandra <sup>3</sup> , Rasnabheda <sup>12</sup> , Chhotaa chaand <sup>17</sup> , Chandrabhaga <sup>18</sup>
3.	English	Serpentina root <sup>4,2,17,18</sup> , Rauwolfia root <sup>17,18</sup> , Serpentine root <sup>18</sup>
4.	Bengali	Chandra <sup>12,16</sup> , Nakuli <sup>12</sup> , Chandar <sup>4</sup> , Chaandar <sup>17,20</sup> , Chhota chand <sup>20</sup> , Gandharasna <sup>12</sup> , Chandara <sup>4,2</sup>
5.	Bihar & Orissa	Dhan-marna or Dhan-barua <sup>16</sup> , Dhanbarua <sup>12,17</sup> , Dhavalbarua <sup>12</sup> , Sanochado <sup>12,17</sup> , Dhanmarva <sup>3,20</sup> , Sanochada <sup>4</sup> , Chandamarva <sup>3,20</sup> , Isargaj <sup>3,20</sup>
6.	Marathi	Amelpodi <sup>12</sup> , Mungusabel <sup>15</sup> , Naaee <sup>15</sup> , Saapand <sup>15</sup> , Adakayi <sup>12,2</sup> , Adkaee <sup>3,20</sup> , Adkai <sup>17</sup> , Chandra <sup>17</sup> , Sayasan <sup>20</sup>
7.	Banaras	Dhavalbarua <sup>3</sup>
8.	Bombay	Harkai <sup>16</sup> , Chandra <sup>16</sup>
9.	Telugu	Patalagandhi <sup>4,16,18</sup> , Patalagani <sup>12,4</sup> , Paatalagaani <sup>20</sup> , Sarpagandhi <sup>17</sup> , Patala garuda <sup>2</sup>
10.	Tamil	Chivan melpodi <sup>16</sup> , Covannamilpori <sup>16,20</sup> , Chivan amelpodi <sup>4</sup> , Sarppaganti <sup>17</sup> , <sup>18</sup> Sivan amelpodi <sup>18</sup> , Civan amalpori <sup>2</sup>
11.	Malyalam	Chuvannavilpori <sup>4</sup> , Chivana avalapori <sup>4,20</sup> , Chivan avelpori <sup>16</sup> , Civan amalpori <sup>2</sup> , Amalpori <sup>17</sup>
12.	Marvadi	Harkaya <sup>4</sup> , Harki <sup>4</sup>

13.	Tulu	Patala-garudada-beru <sup>16</sup>
14.	Gujrati	Amelpodi <sup>20</sup>
15.	Gwalior	Naya <sup>16</sup>
16.	Kannada	Sutranabhi <sup>12,4,18</sup> , Sarpagandhi <sup>4</sup> , Sutranaabhu <sup>17</sup> , Patalagaruda <sup>18</sup> , Sutranavi <sup>20</sup>
17.	Farasi	Chhotachanda <sup>12, 15</sup>

### 3.4.6.2 Habit

It is a glabrous perennial undershrub<sup>4</sup>.

### 3.4.6.3 Habitat

Found in the tropical Himalayas and at moderate altitudes in Sikkim, North Bihar, Patna, Bhagalpur, Bengal, Konkan, Assam, Burma, Shrilanka, Andaman, Pegu, Tenasserim and Deccan Peninsula along the Ghats to Travancore and Ceylon, Java and Malay Peninsula<sup>16</sup>. Found at 4000 feet height of the sea level in moist jungle and shaded areas and now is cultivated in different areas as Dehradun, Lakhnow, Jammu, Indore etc.

### 3.4.6.4 Morphological characters

A perennial, glabrous, under shrub, 15 cm-1mtr high<sup>4</sup>.



**Figure 3.1:** Plant of *Rauwolfia serpentina*

**Leaves** are in whorls 3-4, rarely opposite, ecliptic-lanceolate or obovate acute or acuminate. Light to dark green in color and soft to touch.



**Figure 3.2:** Leaf of *Rauwolfia serpentina*

**Flowers** are white, pink, red or bluish white in color around 3 cm. in size and these are arranged in bunches on the branch.



**Figure 3.3:** Flowers of *Rauwolfia serpentina*

**Fruits** are drupes, pea sized, purple black when ripe, **seeds** ovoid.



**Figure 3.4:** Fruits of *Rauwolfia serpentina*

**Root** pieces are thick, curved and stout, rarely branched, sub cylindrical in shape on breaking it is circular with centripetal lines and externally it is grayish yellow to brown in color.



**Figure 3.5:** Roots of *Rauwolfia serpentina*

#### **3.4.6.5 Microscopical characters**

The transverse section (T.S) of *Rauwolfia* root having outermost multilayered stratified cork composed of alternate bands of 5-10 rows of a small suberized cells and 2-5 rows of big sized lignified cells; phelloderm is parenchymatous embedded with starch grains and small sized twin prismatic crystals of calcium oxalate; phloem is narrow, parenchymatous, traversed with medullary rays, latex cells, calcium oxalate crystals and starch grains; cambium ring is distinct; xylem is lignified, composed of few small sized isolated or radially arranged xylem vessels, tracheids and fibers alternating with uni- or multiserate medullary rays, parenchymatous cells are pitted and embadded with starch grains<sup>4,17,21,22</sup>.

**Powder microscopy** of the plant root shows stratified cork, tracheid and vessels, starch grains, calcium oxalate crystals, xylem fibers and latex cells<sup>4</sup>.

#### **3.4.6.6 Part used**

Root<sup>17,4,18,19,20,21</sup>, leaves<sup>18</sup>

#### **3.4.6.7 Ayurvedic Properties**

Rasa (taste) - Tikta (bitter)<sup>2,17,20,19</sup> Katu (pungent)<sup>17</sup>

Guna (property) - Ruksha (dry)<sup>2,17,19,20</sup> Laghu (light)<sup>17</sup>

Virya (potency) - Ushna (hot)<sup>2,15,19,20</sup>

Vipaka (metabolism) - Katu (pungent)<sup>2,17,19,20</sup>

Prabhava (specific action) - Nidrajanan (sedative)<sup>20</sup>, Kaphavatahar<sup>19</sup>

#### 3.4.6.8 Actions of *Rauwolfia serpentina*

The drug Sarpagandha is given in many disorders like anidra (insomnia), apasmar (epilepsy), bharama (disorientation), javara (fever), krimiroga (worm infestation), medoroga (obesity), unmand (insanity), manasaroga (mental ailments), sula (pain), bhutavadha (influence of evil spirit)<sup>18</sup>. The root of the plant is tikta (bitter), katu (acid), rechak (laxative), kriminashan (anthelmintic), thermogenic and mutral (diuretic) and possesses nidrajanan (sedative) properties. It is extremely used for uchha raktachap (hypertension), and useful in mutrakrichha (strangury), vrana (wounds), udarsula (colic), vertigo, apacha (dyspepsia) and vitiated condition of kapha and vata. The decoction of roots is used to increase uterine contractions. The swarasa of leaves is used in the treatment of eye disorders<sup>17,18</sup>.

#### 3.4.6.9 Dosage

Insomnia: 3-6 gm<sup>2,20</sup>, 1-2 gm<sup>19</sup>

Hypertension: 1-2 gm<sup>2,20</sup>, 0.5-0.75 gm<sup>19</sup>

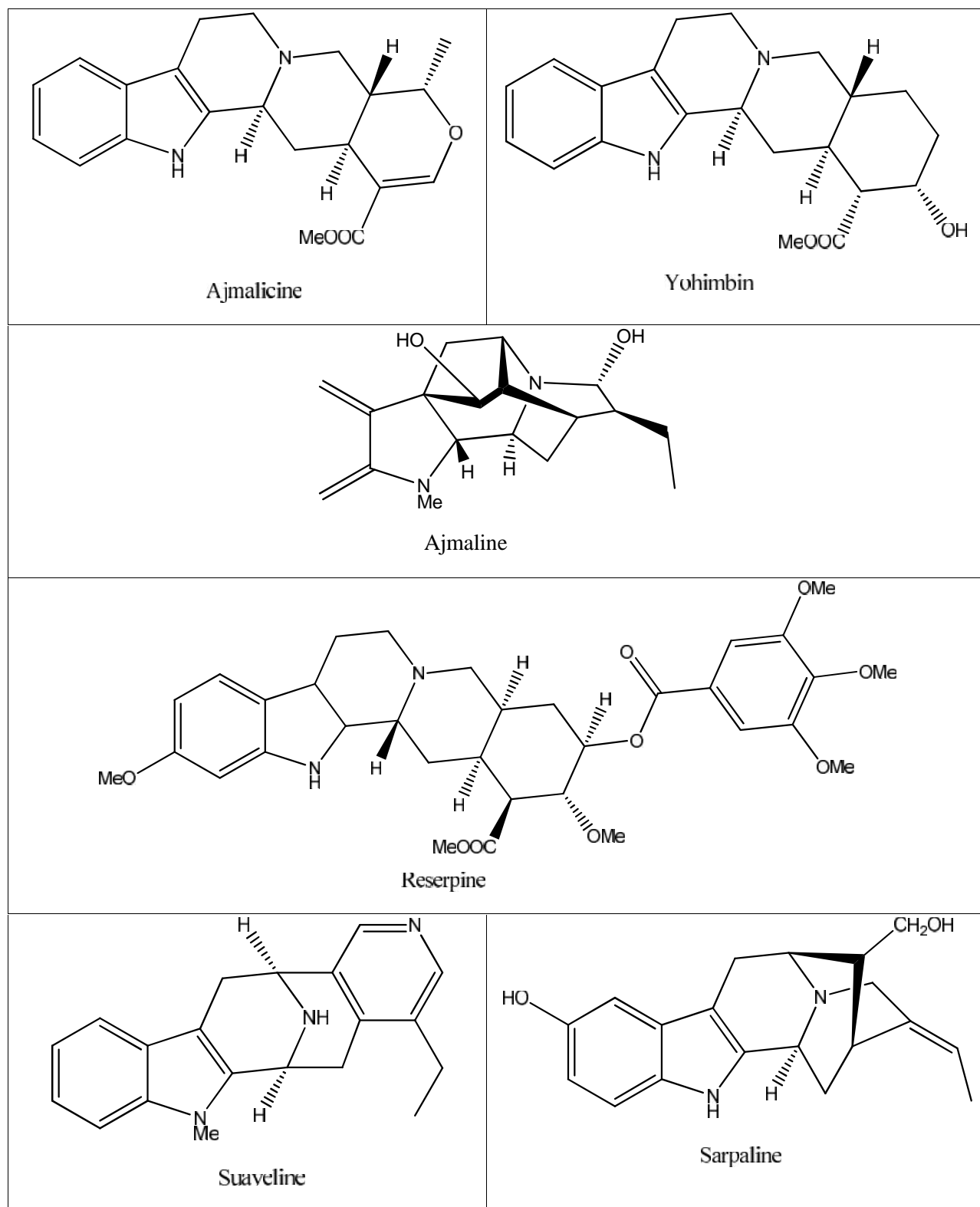
Schizophrenia: 3-6 gm<sup>2</sup>

Mental condition: 1.5-3 gm<sup>19</sup>

#### 3.4.6.10 Chemical constituents

Major constituents are: Reserpine, Rescinnamine, Serpentine, Ajmaline, Ajmalicin, Ajmalicidine, Rauhimbine, Indobinine, Reserpiline, Sarpagine, Serpentine, Serpentinine, Yohimbine, Ajmalimine, Ajmaline, Rauwolfinine (perakenine), Sandwicolidine, Serpinin<sup>2</sup>. The root part of the plant contains another alkaloid named as “ophioxylin” which is crystalline and orange in color. Other constituents that it contains are like salt, resin, gum potassium carbonate, phosphate, starch wax<sup>2,17,19,20,21</sup>.





**Figure 3.6:** Structures of few chemical constituents of *Rauwolfia serpentina*

### 3.4.6.11 Recent studies

#### 3.4.6.11.1 Review Articles

- *Rauwolfia serpentina* popularly used in the treatment of mental illness, snakebite, hypertension etc. The methanolic and aqueous extract of *Rauwolfia serpentina* is used for exclusion of the fetus, diarrhea, dysentery, colic etc. In vitro propagation studies revealed that technique may be a solution for rapid propagation of plant species<sup>23</sup>.

#### 3.4.6.11.2 Pharmacognostical study

- The antibacterial activity of *Rauwolfia sepentina* is reported due to the presence of an alkaloid, reserpine. Reserpine was detected, monitored and quantified by HPTLC and various pharmacognostic parameters were established for their correct identification<sup>24</sup>.
- For the quality assurance and authentication of herbal drugs WHO recognized some protocols for medicinal plants. *Rauwolfia serpentina* having different types of cells like cork cells (contains layers of different cells), parenchymatous cells (some of them are filled with starch grain), vessels, tracheids, xylem parenchyma, wood fibers, xylem rays sieve cells, companion cells which contains starch grains, crystals ( rosette and prismatic type) and some brown color resinous material<sup>25</sup>.
- The roots of different species of *Rauwolfia* are sometimes adulterated or interchanged due to limited knowledge in identification. Pharmacognostic evaluations provide detailed diagnostic and distinctive characters to differentiate the species of *Rauwolfia*<sup>26</sup>.

#### 3.4.6.11.3 Analytical study

- The quantitative phytochemical investigation of roots extracts of *Rauwolfia serpentina* indicates the presence of alkaloids (12.4%), fats (2%), saponin (7.35%). Various GC-MS studies of the n-hexane extract of plant indicates the presence of different 18 compounds which may contribute in the medicinal value of the plant<sup>27</sup>.
- The *Rauwolfia serpentina* is cultivated due to the active ingredients present in it which are having medicinal use. Approximately 50 alkaloids are present in which reserpine is main constituent which act in hypertension. The identification and confirmation of the reserpine and serpentine is done by thin layer chromatography and chemical test. The root and leaves of the plant having crude content is about 0.416 mg/gm and 0.217 mg/gm on dry weight basis. The amount of reserpine and other alkaloids is higher in root fraction as compare to the leaf<sup>28</sup>.

- In this study the plant was analysed for phytoconstituents present in it. The result shows the presence of steroids, glycosides, carbohydrates, flavanoids, saponins, alkaloids & triterpenes which are biologically active. *Rauwolfia serpentina* (Linn.) is an excellent source of these bioactive compounds. Hence the plant is used for the extraction of useful drugs. The extract of the plant provided some phytochemical basis for the ethnomedicinal use in the treatment and prevention of infections<sup>29</sup>.
- The flavonoidal content of *Rauwolfia serpentina* was isolated from the alcoholic extract of the plant. The structure of isolated compound was determined by using spectroscopic and chromatographic techniques and determined as, Quercetin<sup>30</sup>.
- *Rauwolfia serpentina* is medicinally renowned herbal plant in both Ayurvedic and western system of medicine and it constitute Reserpine (an important indole alkaloid) which is reported to have tranquilizing and anti hypertensive activity. In this study HPTLC of Reserpine has been developed for detection, monitoring and quantification. It was found at Rf 0.43<sup>31</sup>.
- The spectrophotometric method is developed for determination of Rauwolfia alkaloids like reserpine, ajmalicine, ajmaline and yohimbine. It involves oxidation of alkaloid by Fe<sup>+3</sup> and subsequent complexation of Fe<sup>+2</sup>. This method is applied for determination of reserpine in tablet<sup>32</sup>.

#### 3.4.6.11.4 Pharmacological study

- **Antioxidant activity**

The ethanolic extract of *Rauwolfia serpentina* root powder was screened in order to recognize its effects on oxidative stress and free radicals. The antioxidant activity was measured by FRAP (ferric reducing ability of plasma or plants). This shows positive results in treating the ill effects of overproduction of free radicals<sup>33</sup>.

The methanolic extract of the leaves of *Rauwolfia serpentina* are investigated for their antioxidant activities, phytochemicals and nutrient composition. Various in-vitro method and HPLC studies are done for the evaluation process. The results provided an substitute of utilizing Rauwolfia leaf as easily available source of natural oxidant inhibitors in cosmetic, food and pharma industry<sup>34</sup>.

The aqueous and methanolic extracts of different medicinal herbs were screened for anti-diabetic and antioxidant activity. The different methods like folin-ciocalteu's for phenolic

content, DPPH and H<sub>2</sub>O<sub>2</sub> for antioxidant activity and in-vitro glucose diffusion and alpha-amylase inhibition assay for anti-diabetic activity. In which the highest alpha-amylase inhibition found in methanolic extract of *Rauwolfia serpentina* and highest diffusion rate of glucose was found in aqueous extract of *Rauwolfia serpentina*<sup>35</sup>.

- **Anti-diabetic activity:**

The methanolic extract of the *Rauwolfia serpentina* is evaluated to find out the arteriosclerosis, atherogenic dyslipidemia and glycosylation indices in alloxan-induced type 1 diabetic mice. They are given with 14 days treatment with alloxan monohydrate and the result obtained shows the curative potential of methanolic extract in decreasing the danger of arteriosclerosis, atherogenic dyslipidemia, and glycosylation in alloxan-induced diabetic mice<sup>36</sup>.

- **The antibacterial activity**

The bacterial inhibition activity of the roots and leaves of *Rauwolfia serpentina* was studied against *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli* by disk diffusion method. The study was done by methanolic and chloroform extract of the drug. The study shows that the methanolic extract of the roots of *Rauwolfia serpentina* has much more potential to inhibit the growth of test organism as compare to the chloroform extract of roots and leaf of the plant<sup>37</sup>.

- **Hypolipidaemic activity**

The root powder of *Rauwolfia serpentina* was investigated to find out its use in controlling hyperlipidaemia, which is the root cause of cardiovascular diseases existing globally. A twelve day trial was done on the rabbits (test and control) with oral administration of *Rauwolfia serpentina* powder. Then the blood serum of the animal is estimated for results. This study shows that *Rauwolfia serpentina* root powder was useful in lowering triglycerides, cholesterol and LDL. This helps in treating atherosclerosis and is a potent hypolipidemic agent which has no toxic effects on liver and cardiac functioning<sup>38</sup>.

- **Inhibition of ACE**

The effect of *Rauwolfia serpentina* leaves (aqueous extract) and *Allium sativum* cloves (aqueous extract) on sheep kidney and lung ACE were studied by using enzyme assay. The medicinal plants which are reducing blood pressure were used in conducting the

study in systematic order. The two plants likely have some possible mechanism by which *Allium sativum* exert renoprotective properties could be through inhibition of ACE activity and *Rauwolfia serpentina* is effective in treating liver disease, cancer and mental illness also<sup>39</sup>.

- **Hepatoprotective activity:**

The aqueous ethanolic extract (AET) of rhizome of *Rauwolfia serpentina* shows the hepatoprotective activity against the paracetamol induced hepatic damage in rats. The AET acts against the hepatotoxicity by hepatic cell regeneration, cell membrane stabilization and activating the anti-oxidative enzymes<sup>40</sup>.

- **Hyperglycemic activity:**

The methanolic extract of roots of *Rauwolfia serpentina* shows the hyperglycemic, haematinic and antioxidant activity against alloxan - induced diabetic mices. The present work conclude that the extract significantly up-grading the antioxidant enzyme system and ameliorate the haematnic, antioxidant and hyperglycemic disfunctioning of the body due to diabetes<sup>41</sup>.

- **Anti diarrhoeal activity**

The methanolic extract of leaves of the plant *Rauwolfia serpentina* possesses antidiarrhoeal action. The 200 mg/kg and 400 mg/kg dose of the extract was given to the mice. The antidiarrhoeal potential was studied against the castor oil induced diarrhea in mice and enterpooling in mice. The methnolic extract showed a considerable reduction in the weight of the faeces as compare to the control group<sup>42</sup>.

- **Anti hypertension**

*Rauwolfia serpentina* has been used in India for several years to treat a variety of diseases including hypertension, epilepsy, insanity, insomnia and hysteria. A variety of diseases have a common denominator by which they all relieved symptomatically by a sedative drug “*Rauwolfia*”. Various clinical trials have been reported for the hypertensive effect of drug<sup>43</sup>.

### 3.4.6.12 Reported formulation

Sarpagandhadi churna<sup>2,17,19,20</sup>, Sarpagandha yoga<sup>17,19,20</sup>, Sarpagandha ghanvati<sup>17</sup> Sarpagandha vati<sup>18</sup> (Maheshwari vati)<sup>19</sup>

### 3.4.6.13 Marketed products

#### Saragandhadi churna

**Rogadhikara:** Hypertension/ high blood pressure

**Dose:** 1-2 gm along with honey as directed by the physician<sup>44</sup>

**Warning:** Do not take this product without the consent of your Ayurvedic Physician.

#### Sarpagandhadi ghanvati

**Rogadhikara:** It is used in the treatment of lack of adequate sleep, hypertension and dizziness.

**Dosage:** (375 mg) – 1 tablet at night or as directed by Ayurvedic doctor<sup>45</sup>.



**Figure 3.7:** Marketed products of *Rauwolfia serpentina*

**CHAPTER IV**  
**RATIONALE AND SCOPE OF STUDY**

**4.1 Rationale and Scope of Study**

This work is conducted to evaluate the comparison of phytoconstituents present in the wild & cultivated variety of *Rauwolfia serpentina*. *Rauwolfia serpentina* is a very significant medicinal herb and used as medicine for variety of disorders like hypertension, fever etc. however; it is one of the endangered species. Due to extinction of species, it is necessary to identify the correct source of plant from which high concentration of active constituents can be obtained from minimum crude drug. This will help us to save our wild resource of *Rauwolfia serpentina* and help to incorporate changes for high concentration of drug in cultivated species if necessary.

## CHAPTER V

### OBJECTIVE OF THE STUDY

#### 5.1 Aim

Comparative Pharmacognostic & Phytochemical Evaluation of Cultivated and Wild variety of *Rauwolfia serpentina* (Apocynaceae)

#### 5.2 Objectives

- Collection of the *Rauwolfia serpentina* roots of cultivated and wild variety.
- Authentification of roots of *Rauwolfia serpentina*
- Pharmacognostical studies of *Rauwolfia serpentina*
- Analytical studies of root
- Qualitative phytochemical study
- Tannin estimation
- Total Phenolic Content
- Thin Layer Chromatographic study
- High Performance Thin Layer Chromatography
- In vitro Antidiabetic study
- In vitro Antioxidant study
- Antimicrobial study



**CHAPTER VI**  
**MATERIALS AND RESEARCH METHODOLOGY**

**6.1 List of Equipment used**

**Table 6.1 List of equipments**

<b>S. No.</b>	<b>Material</b>
1	Weighing balance
2	Grinder
3	Sieves
4	Plastic containers
5	Beakers
6	Crucible
7	China dish
8	Simple Microscope
9	Dissecting Microscope
10	Electronic Microscope
11	Hot plate
12	Water bath
13	Hot air oven
14	Rotary vacuum evaporator
15	Magnetic stirrer
16	Ultra centrifuge
17	Measuring cylinders
18	TLC Plates
19	TLC Chamber
20	HPTLC
21	Colorimeter
22	UV Chamber
25	UV spectrophotometer
26	Micropipettes
27	Volumetric flasks

## 6.2 Chemical used

**Table 6.2 List of Chemicals**

S. No.	Material	S. No.	Material
1	Sarpagandha ( <i>Rauwolfia serpentina</i> )	26	Sodium picrate
2	Methanol	27	Pyridine
3	Petroleum ether	28	Sodium nitroprusside
4	DPPH	29	Glacial acetic acid
5	Acarbose	30	Benzene
6	Chloroform	31	Bromine
7	Hydrochloric acid	32	Acetic acid
8	Ferric chloride	33	Potassium permanganate
9	Lead acetate	34	Potassium dichromate
10	Sodium hydroxide	35	Fehling's A & Fehling's B
11	Copper sulphate	36	Benedicts reagent
12	Ninhydrin	37	Magnesium turnings
13	Gallic acid	38	Sodium bicarbonate
14	Tannic acid	39	Starch
15	Gelatin	40	Wagner's reagent
16	Hager's reagent	41	Mayers reagent
17	Dragandroff's reagent	42	Silica gel g
18	Picrolinic acid solution	43	Vanillin
19	Conc. Sulphuric acid	44	Folin densin reagent
20	Sudan III	45	Phloroglucinol
21	Iodine	46	Nutrient Agar
22	Ethanol	47	Toluene
23	E coli	48	Ethyl acetate
24	Alpha- naphthol	49	Diethylamine
25	Ascorbic acid	50	Formic acid

### 6.3 Research Methodology

**Table 6.3: Research Methodology**

Sr. No.	Methodology
1	<b>Selection of crude drug</b>
2	<b>Literature review</b>
3	<p><b>Collection of crude drug</b></p> <p>The root sample of cultivated <i>Rauwolfia serpentina</i> was collected from Herbal Garden and Herbarium, Research Institute in Indian System of Medicine, Joginder Nagar and the wild sample was also collected from the Joginder Nagar with the help of local residents.</p>
4	<p><b>Authentication</b></p> <p>The authentication of the root sample of <i>Rauwolfia serpentina</i> was done from Department of Botanical &amp; Environmental Sciences Guru Nanak Dev University, Amritsar (India)</p>
5	<p><b>Pharmacognostical work</b></p> <ul style="list-style-type: none"> <li>• Macroscopic study</li> <li>• Microscopic study</li> <li>• Powder characters</li> </ul>
6	<p><b>Analytical study</b></p> <ul style="list-style-type: none"> <li>• Foreign matter</li> <li>• LOD</li> <li>• Ash value <ul style="list-style-type: none"> <li>Acid insoluble ash value</li> <li>Water soluble ash value</li> </ul> </li> <li>• Extractive values <ul style="list-style-type: none"> <li>Water soluble extractive values</li> <li>Alcohol soluble extractive values</li> <li>Ether soluble extractive values</li> </ul> </li> </ul>
7	<p><b>Qualitative phytochemical screening of roots of <i>Rauwolfia serpentina</i></b></p> <ul style="list-style-type: none"> <li>• Tannin estimation</li> <li>• Total Phenolic Content</li> </ul>
8	<p><b>Chromatographic studies</b></p> <ul style="list-style-type: none"> <li>• Performance of TLC &amp; HPTLC study</li> </ul>
9	<p><b>Pharmacological activity</b></p> <ul style="list-style-type: none"> <li>• Antimicrobial study</li> <li>• In-vitro Antioxidant Activity</li> <li>• In-vitro Antidiabetic Activity</li> </ul>

## CHAPTER VII

### EXPERIMENTAL WORK

#### 7.1 Material and Methods

##### 7.1.1 Identification of the plant

*Rauwolfia serpentina* was identified by referring its taxonomical and morphological characters mentioned in different texts.

##### 7.1.2 Collection of plant material

Root sample of cultivated *Rauwolfia serpentina* was collected from Herbal Garden and Herbarium, Research Institute in Indian System of Medicine, Joginder Nagar and the wild sample was also collected from the Joginder Nagar with the help of local residents.

##### 7.1.3 Authentication of the plant

The collected crude drugs were submitted in Department of Botanical & Environmental Sciences at Guru Nanak Dev University, Amritsar (India) for authentication purpose.

##### 7.1.4 Preparation of powder

The dried roots of *Rauwolfia serpentina* were coarsely powdered by using grinder.

##### 7.1.5 Storage of plant material

The dried roots were stored in the air tight plastic containers and the rest of powdered material was kept in separate plastic containers with suitable label and stored in dark place.

#### 7.2 Pharmacognostic study

##### 7.2.1 Macroscopic characters

**Size:** The measure of the length width and thickness of the sample is done by graduated ruler.

**Color:** Samples were examined under diffused day light.

**Surface:** The material was touched to determine it was smooth or rough.

**Odor:** The plant material was powdered then its odor was determined.

**Taste:** Small amount of powdered material was mixed with water, than a drop was tasted.

**Fracture:** The material was bend or ruptured.

**Shape:** shapes were examined through magnified glass.

##### 7.2.2 Microscopic characters

**Entire material:** The transverse section of the dried root was taken by hand and mounted on the slide with reagent phloroglucinol and conc. hydrochloric acid and examined under binocular

microscope under 10X and 45X and images were taken with the help of HTC 13 megapixel camera

#### **Powdered material:**

The powdered material was taken and mounted on the microscopic slides with different reagents and examined under binocular microscope (10X) and the images were taken with the help of digital camera.

### **7.3 Analytical study**

#### **7.3.1 Foreign Matter**

Foreign matter is the substance in the sample other than the drug sample. Foreign matter consisting of other plant part which is not taken as drug of and other material like insects, moulds, animal faecal matter and other contamination like soil, stone etc.

#### **Determination of foreign matter**

- Weigh 100-500 gm of sample or minimum quantity as prescribed in monograph.
- Spread a thin layer of plant material on a sheet of paper.
- Examine the foreign matter by inspecting naked eye or using lens (6X).
- Separate the foreign matter and weigh.
- %age of the foreign matter is then calculated.

#### **7.3.2 Determination of Total Ash Value**

- Incinerate about 2-3gm of coarsely powdered drug in tare platinum and silica dish at 450<sup>0</sup> temperature until it is free from carbon, then cool and finally weigh it.
- The %age of ash value is calculated with reference to air dried drug.

#### **7.3.3 Determination of Acid Insoluble Ash Value**

- 25 ml of the dilute hydrochloric acid is added to the ash and then boiled it for five minutes.
- Filter the content by means of ash less paper and wash them with hot water
- Insoluble matter is collected and ignited to a constant weight
- %age acid insoluble ash is calculated with reference to the air dried drug.

#### **7.3.4 Determination of Water Soluble Ash Value**

- 25 ml of water was added to ash and boiled it for 5 min.
- Wash the content with warm water and insoluble matter was collected on ash less filter paper in a Gooch crucible.

- Ignite for 15 min at 450<sup>0</sup> temperature.
- Water soluble ash is obtained by subtracting insoluble matter from the weight of ash.
- %age of water soluble ash is calculated with reference to air dried sample.

### **7.3.5 Determination of Alcohol Soluble Extractive Value**

- Weigh 5 gm of sample and macerate in 100 ml of alcohol in a conical flask (closed) for 24 hrs. (Shaking frequently 6 hours and allow standing for 18 hours)
- Filter the solvent rapidly without any loss.
- Evaporate 25 ml of solvent in tared evaporating dish on water bath at 105<sup>0</sup> temperature to the constant weight.
- The %age of alcohol soluble extractive value is Calculate with reference to air dried drug.

### **7.3.6 Determination of Water Soluble Extractive Value**

- Accurately weigh 5 gm of drug and macerate in 100 ml of chloroform water in a closed conical flask for 24 hrs. (Shaking frequently 6 hrs. and allowing standing for 18 hrs.)
- Filter the solvent rapidly without any loss.
- Evaporate 25 ml of the solvent in tared evaporating dish on water bath at 105<sup>0</sup> temperature to the constant weight.
- The %age of alcohol soluble extractive value is calculated with reference to air dried drug.

### **7.3.7 Determination of Moisture Content (LOD)**

- Accurately weigh about 10 gm of coarsely powdered drug in a tared evaporating dish.
- Dry for 5 hrs at 105<sup>0</sup> and then weigh.
- Drying and weighing is continued till 1 hr interval until the difference between two successive weighing corresponds to not more than 0.25 per cent.
- When two consecutive weighing reaches the constant weight after drying for 30 minutes and cooling for 30 minutes in desiccators, shows not more than 0.01 g difference.

## 7.4 Preliminary phytochemical investigation

**Table 7.1: Phytochemical testing for various compounds**<sup>21,22</sup>

S. No.	Test for		Method/ amount	Result
1	Alkaloids	Mayer's test	2-3 ml extract + drops of Mayer's reagent	ppt.
		Dragendorff's test	Extract + few drops of Dragendorff's reagent	Orange brown ppt.
		Hager's test	Extract + Hagers's reagent	Yellow ppt.
		Wagner's test	Extract + Wagner's reagent	Reddish brown ppt.
		Picrolonic acid test	Extract + Picrolonic acid test	Yellow ppt.
		Tannic acid test	Extract + Tannic acid	Buff color ppt.
2	Carbohydrates	Molisch's test	Extract+ Alpha nepthol (shake) + H <sub>2</sub> SO <sub>4</sub>	Violet ring is formed
		<b>Reducing sugars</b> Fehling's test	Mix equal Fehling's A and Fehling's B (boil) + extract (heat 5-10 min.)	Yellow followed by brick red ppt.
		Benedict's test	Mix equal extract and Benedict's reagent (heat for 5min.)	Green /yellow / red color appears
3	Glycosides	<b>Cardiac glycosides</b> Baljet's test	Thick section of sample + Sodium picrate	Yellow to orange color
		Legal's test	Extract + Pyridine + Sodium nitroprusside	Pink to red color
		Test for deoxy sugars (Keller)	Extract + Glacial acetic acid + FeCl <sub>3</sub> + Conc. H <sub>2</sub> SO <sub>4</sub>	Reddish brown color at junction

		killiani test)		and upper layer bluish green
		<b>Anthraquinone glycosides</b> Borntrager's test	Extract + dil. H <sub>2</sub> SO <sub>4</sub> (boil and filter) + equal benzene or chloroform(shake) separate organic solvent + ammonia	Pink or red color ammonical layer
		<b>Saponin glycosides</b> Foam test	Extract /dry powder + water (shake)	Persistent foam
		<b>Cynogenetic glycosides</b> Guignard reaction or Sodium picrate test	Soak a filter paper in Picric acid and sodium carbonate(dry) exposed filter paper with moisten drug powder	Brick red to maroon color of filter paper
		<b>Coumarin glycosides</b>	Odor	Aromatic odor
<b>4</b>	<b>Flavanoids</b>	Shinoda test	Powder/extract+95% ethanol/t-butyl alcohol + drops of conc. HCl+ 0.5g magnesium turning	Orange, pink, red, to purple color
			Residue/ extract + Lead acetate solution	Yellow color ppt.
			Test solution + Zinc and HCl	Pink to red color appeared.
<b>5</b>	<b>Tannins and phenolic acids</b>		Extract + few drops of FeCl <sub>3</sub>	Deep blue-black color
			Extract + few drops of Gelatin	White ppt.



		solution	
		Extract + few drops of Bromine water	Decoloration of bromine water
		Extract + few drops of Acetic acid solution	Red color
		Extract + few drops of Dil. Potassium permanganate solution	Discoloration
		Extract + few drops of Potassium dichromate	Red ppt.

### 7.5 Estimation of Total Phenolic content (TPC)

**Standard gallic acid solution:** Accurately weighed quantity of gallic acid is dissolve into distilled water to get the concentration of 1mg/ml.

- Spectrophotometric methods are used to determine the TPC in which 1 ml of sample is mixed with 1 ml of Folin- Ciscaltue's phenol reagent.
- Later on, 1 ml of Na<sub>2</sub>CO<sub>3</sub> solution (7%) is added to it which is followed by the addition of 1.3 ml of de-ionized distilled water.
- Mix the solution thoroughly.
- Then it is kept in the dark for ninety minutes at 23<sup>0</sup>C and lastly absorbance is recorded at 750 nm.
- TPC is determined by extrapolation of the standard calibration curve.
- The phenolic compound estimation is carried out in triplicate manner<sup>47</sup>.

### 7.6 Estimation of Total Tannic Acid content

**Standard tannic acid solution:** Accurately weighed quantity of tannic acid is dissolved in distilled water to get the 1 mg/ml conc.

#### Extraction of Tannin:

- 0.5 g of the powdered material is transferred to conical flask (250 ml) and 75 ml of water is added to it. Then the flask is heated and boiled gently for 30 minutes. The sample is centrifuge at 2,000 rpm for 20 minutes and then supernatant is collected in 100 ml volumetric flask & make up the volume.
- Then 1 ml of the extract is transferred to a 100 ml volumetric flask containing 75 ml water. After that 5 ml of Folin-Denis reagent, 10 ml of sodium carbonate solutions is

added to it and diluted to 100 ml with water. Shake well and after 30 minutes, read the absorbance at 700 nm.

- The blank is prepared with distilled water<sup>48</sup>.

## 7.7 TLC

### Preparation of test sample:

- Coarsely powder of different samples of *Rauwolfia serpentina* is extracted separately in soxhlet apparatus using ethanol for 6 hours.
- The extracts are then concentrated using water bath evaporator.

**Saturation of TLC chamber:** The TLC chamber is saturated with solvent system for 30 min.

### TLC plates:

#### Application of spots:

- Single spot
- Band spot

**Development of plates:** The plates are development in TLC jar and development for 7 cm and allowed to air dry.

**Visualization:** TLC plates are observed visible and under UV light<sup>49,50,51</sup>.

### Calculation of retardation factor (Rf) value:

Formula for using Rf value:

$$\text{Rf value} = \frac{\text{Distance travelled by the spots}}{\text{Distance travelled by solvent front}}$$

## 7.8 HPTLC

### Apparatus

- Instrument: CAMAG Linomat 5 "Linomat5\_180745" S/N 180745 (1.00.12)
- Syringe: 100 µL
- HPTLC plates: 6.0 x 10.0 cm

### Application parameters

- Spray gas: inert gas
- Sample solvent type : methanol
- Dosage speed : 150 nl/s
- Predosage volume: 0.2 µl

## Preparation of Standard and Sample Solutions

- Standard reserpine solution is prepared of 1 mg/ml concentration by dissolving 10 mg of reserpine in 10 ml of methanol.
- Sample solution is prepared by dissolving 10 mg of methanolic extract of sample in 10 ml of methanol to get the concentration of 1 mg/ml.

## Procedure

- Precoated aluminium plates are used to carry out the HPTLC procedure.
- About 2 µL of sample is applied on the silicagel 60 F plate and the spotting is done in band form.
- Each band is of 10.0 mm in length and different 3 bands are spotted on a plate.
- The scanning speed of the instrument is 20 mm/s
- The plate are dried and scanned at 254 nm in absorbance mode.
- Amount of reserpine is determined by using calibration curve which is plotted between conc. & area of standard reserpine<sup>52</sup>.

## 7.9 Antimicrobial study:

### 7.9.1 Processing of the plant

- Healthy roots of *Rauwolfia serpentina* are collected and washed properly.
- Roots are then shade dried and grounded by using grinder.
- The powder of roots is extracted in both distilled water and methanol; 10 gm of plant powder is extracted in respected solvent using soxhlet extractor.
- Then the extract is collected and concentrated till dryness.
- The stock solution is prepared of 1 mg/ml concentration.

### 7.9.2 Antimicrobial assay

- Agar well diffusion method is used to evaluate the microbial inhibitory activity of the sample.
- The entire test organism is inoculated on MHB for 8 hours.
- With the help of sterilize cotton swabs, isolates are seeded on MHA plates.
- Sterilized borer is used to make bore of 4 mm diameter on agar surface.
- An amount of 100 µL of the sample extract (test) and sterilized distilled water (control) are poured into separate wells in separate plates with standard antibiotic disc.

- Then the plates are incubated for 48 hours at 37<sup>0</sup>C temperature.
- Experiment is done in triplicates<sup>52</sup>.

### 7.10 In vitro evaluation of antioxidant activity

**Preparation of the sample:** About 40 gm of the root powder of *Rauwolfia serpentina* is soaked in methanol whole night. In the next morning, extract is filtered through whatman filter paper. Filtrate obtained is then concentrated till dryness. Then the stock solution is prepared of 1 mg/ml concentration.

- 700 µL of sample is added with the similar volume of methanolic solution of a 100 µM DPPH.
- After this the solution is shaken vigorously and left in the dark at room temperature for 20 minutes.
- Then the absorbance is recorded at 515 nm<sup>53</sup>.

$$\text{Inhibition (I \%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} * 100$$

### 7.11 In vitro anti-diabetic activity

**Preparation of the sample:** About 40 gm of the root powder of *Rauwolfia serpentina* is soaked in methanol whole night. In the next morning, extract is filtered through whatman filter paper. Filtrate obtained is then concentrated till dryness. Then the stock solution is prepared of 1 mg/ml concentration.

- An amount of 500 µl of each plant extract, sodium phosphate buffer (0.2 M, containing - amylase solution) and starch solution (1%) are taken in a test tube and incubated for 10 minutes at 37°C.
- Add 500 µl of sodium chloride to each tube at 5s intervals after incubation and further 1000 µl of DNSA is added to stop the reaction process.
- Then test tubes are kept in boiling water for 5 minutes and cooled.
- Further it is diluted with 10 ml distilled water and absorbance is recorded at 540 nm<sup>54</sup>.

$$\text{Inhibition (I \%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{control}}} * 100$$

## CHAPTER VIII

### RESULT AND DISCUSSION

#### 8.1 Literature review

The plant *Rauwolfia serpentina* is used in common practice since antiquity. The first written reference of the plant was found in the Samhita kala. Acharya Charak in his Charak Samhita has mentioned Nakuli plant which is one of the synonym of Sarpagandha. In Sushruta Samhita, the uses of the plant are explained in Aparajita gana. It is indicated in metal disorders. Other Acharyas have explained the plant by different synonyms like: Nakuli, Katushna, Vishhanti, Dhavalbaruaa etc. The plant has tikta (bitter) taste and has ushna (hot) potency and having nidrajanan (hypnotic) effect on body.

According to the principles of Dravyaguna, tikta (bitter) rasa (taste) is composed of aakash (sky/vacuum) and vayu (air) mahabhuta and ruksha (dry) guna has the dominance of agni (fire) and vayu (air) mahabhuta. Due to the presence of agni (fire) and vayu (air) mahabhuta, it has ushna (hot) virya. Moreover it is kaphavatahar and pitta vardhak due to its ushna virya. Due to its tikta rasa it is used as krimighna (anthelmintic), aampachan (digestive) and jwaraghana (reduce fever).

It contains many chemical constituents like ajmalicine, ajmalidine, rohuimbine, indobinine, reserpiline, reserpine, serpagine, serpentine, serpentinine, yohimbine, ajmalimine, ajmaline, rauwolfinine, serpinine etc. Reserpine (an indole alkaloid) is the antihypertensive principle having tranquillizing property. The root powder is generally used for different disorders like: anidra (insomnia), rakt chapa vridhhi (hypertention), schizophrenia, apsmara (epilepsy), unmanda (insanity), siragata vata, bhrama, shula (pain) etc.

#### 8.2 Authentication

The sample of *Rauwolfia serpentina* (Apocynaceae) is authenticated by Department of Botanical and Environmental Sciences Guru Nanak Dev University, Amritsar (India).

##### 8.2.1 Details of Authentication

**Table 8.1 Details of Authentication**

Botanical Name	Family	Place	Ref. No.
<i>Rauwolfia serpentina</i>	Apocynaceae	GNU, Amritsar	1335

## 8.3 Pharmacognostic Study

### 8.3.1 Macroscopic Studies

**Table 8.2: Macroscopic characters of *Rauwolfia serpentina* (wild & cultivated)**

Sr. no.	Contents	Observations	
		<i>Rauwolfia serpentina</i> (cultivated)	<i>Rauwolfia serpentina</i> (wild)
1	Colour	Grayish brown	Grayish yellow
2	Odour	Slight odour	Slight odour
3	Taste	Bitter	Bitter
4	Shape	Longitudinal sub-cylindrical cut pieces	Longitudinal sub-cylindrical cut pieces
5	Surface	Irregular	Irregular
6	Fracture	Short	Short
7	Dimensions	Pieces of 8-15cm*0.5-2.0cm	Pieces of 8-15cm*0.5-2.0cm
8	Touch	Hard	Hard

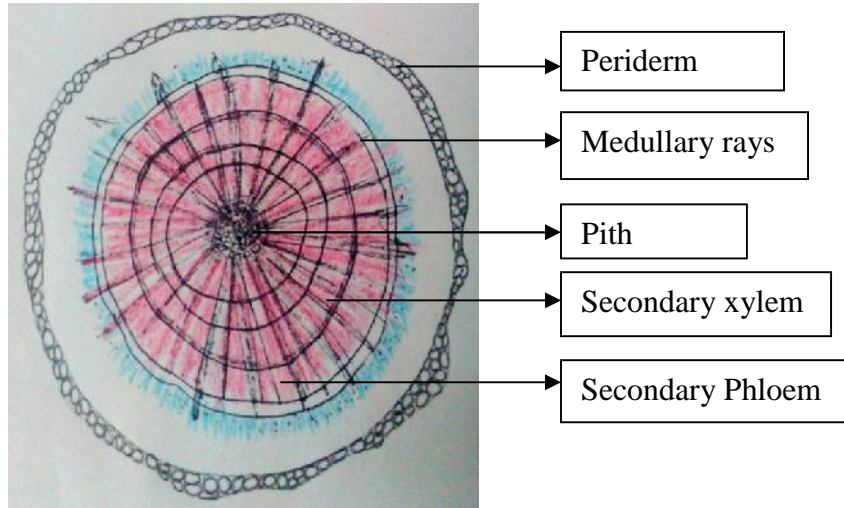


**Figure 8.1:** Roots of Sarpagandha (cultivated)

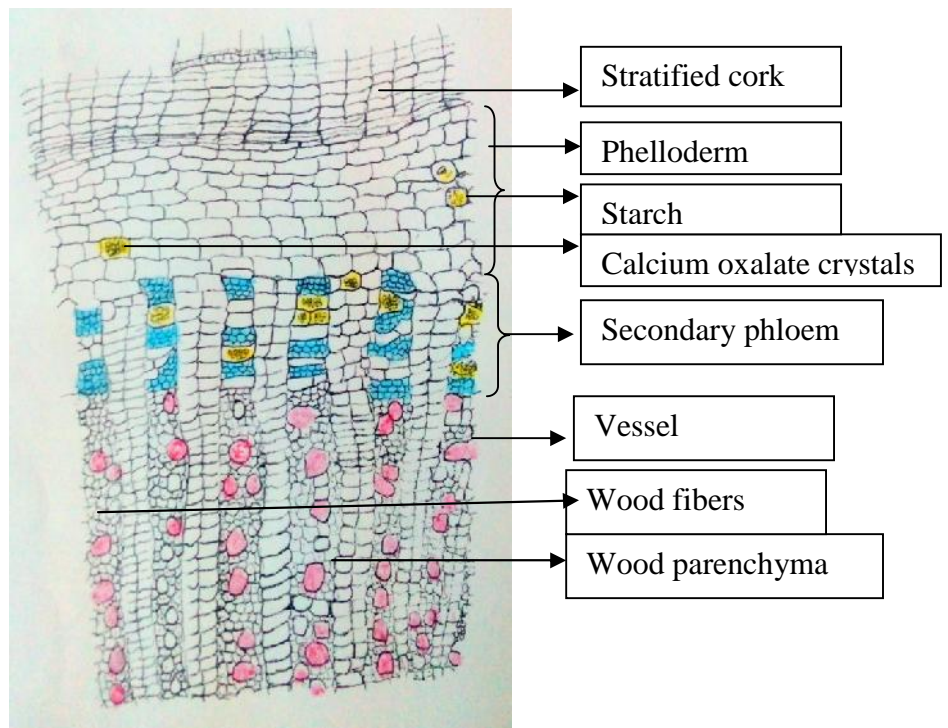


**Figure 8.2:** Roots of Sarpagandha (wild)

### 8.3.2 Microscopic Studies

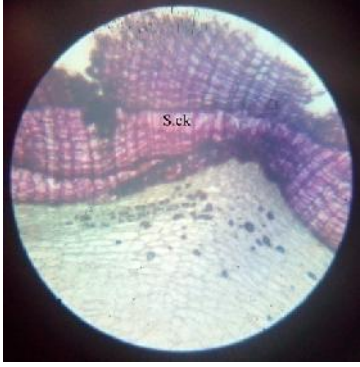
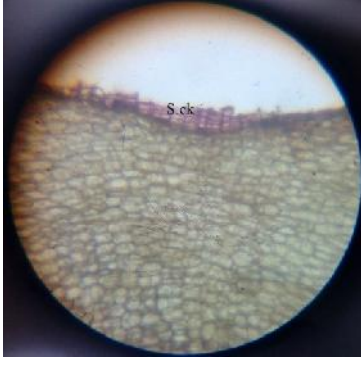
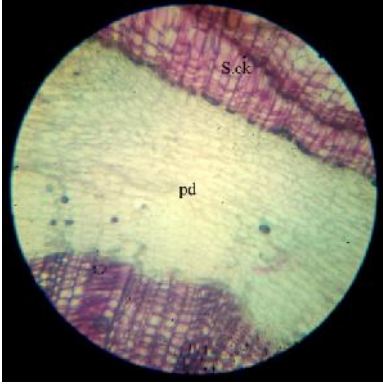
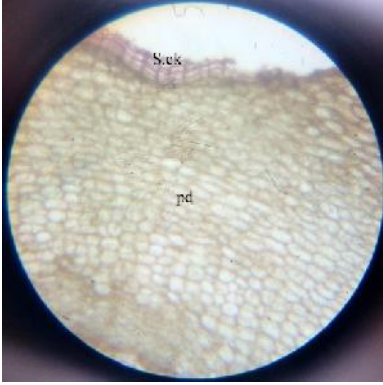
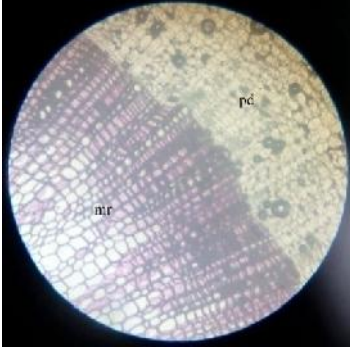



**Figure 8.3:** Diagrammatic sketch of T.S. of Rauwolfia root

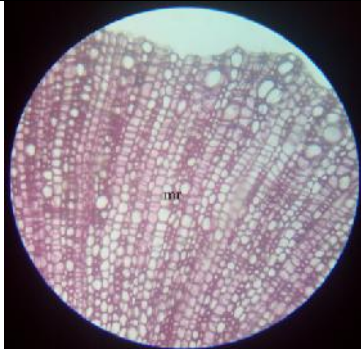

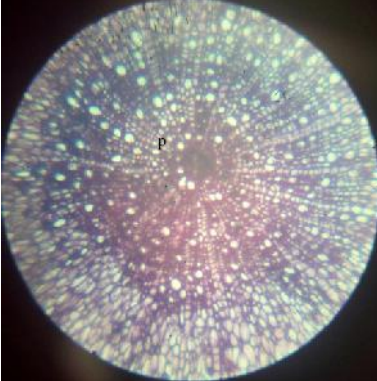
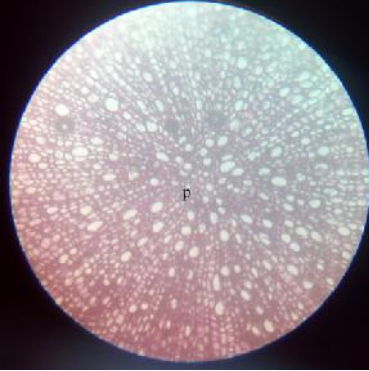


**Figure 8.4:** Diagrammatic sketch of T.S. of Rauwolfia root

**8.3.2.1 Microscopy of *Rauwolfia serpentina***

Cultivated	Wild
	
<p><b>Figure 8.5:</b> Outermost S.ck (stratified cork cells) layer of root of Sarpagandha root</p>	<p><b>Figure 8.6:</b> Outermost S.ck (stratified cork cells) layer of root of Sarpagandha root</p>
	
<p><b>Figure 8.7:</b> pd (Phelloderm cells) between upper and lower cells of Sarpagandha root</p>	<p><b>Figure 8.8:</b> pd (Phelloderm cells) between upper and lower cells of Sarpagandha root</p>
	



<p><b>Figure 8.9:</b> pd (Phelloderm cell) layers with small portion of mr (medullary rays) of Sarpagandha root</p>	<p><b>Figure 8.10:</b> pd (Phelloderm cell) layers with mr (medullary rays) of Sarpagandha root</p>
	
<p><b>Figure 8.11:</b> mr (Medullary) region of Rauwolfia root</p>	<p><b>Figure 8.12:</b> mr (Medullary) region of Rauwolfia root</p>
	
<p><b>Figure 8.13:</b> Centre p (pith) portion of root of Sarpagandha</p>	<p><b>Figure 8.14:</b> Centre p (pith) portion of root of Sarpagandha</p>

The T.S. of *Rauwolfia serpentina*, wild and cultivated variety is shown in these figures which revealed that both wild and cultivated plants having moreover the same microscopic characters with slight changes in the size and shape of the cells. Number of layers of cork cells in wild variety is more as compare to cultivated; the phalloderm cells are compact in cultivated variety towards the phloem region.

**8.3.2.2 Powder of *Rauwolfia serpentina***

Cultivated	Wild
	

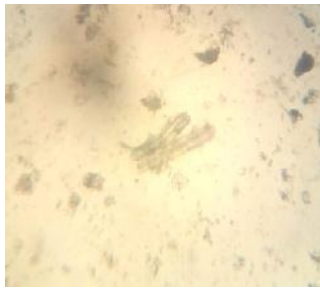
**Figure 8.15:** Roots powder of *Rauwolfia serpentina*

**Figure 8.16:** Root powder of *Rauwolfia serpentina*

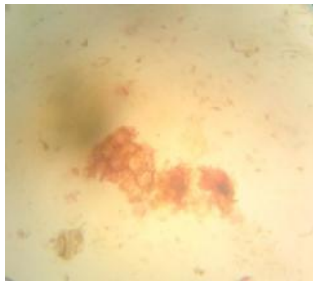
a



b



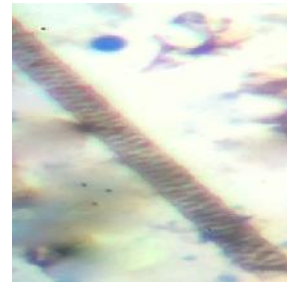
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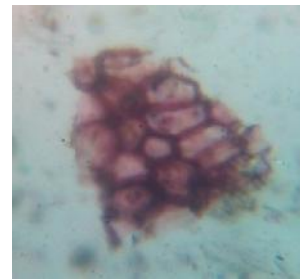
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e



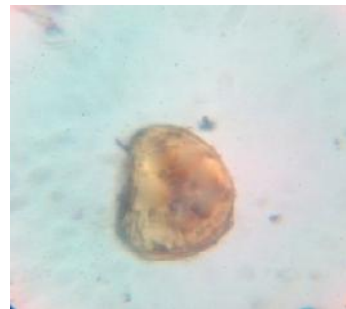
f



g



h



**Figure 8.17:** a: fiber, b: xylem cells, c: cork cells, d: parenchyma cells, e: vessel, f: parenchyma cells, g: spiral vessel, h: resin present in powder

## 8.4 Identity, Purity and Strength of *Rauvolfia serpentina*

### 8.4.1 Determination of Foreign Matter

**Table 8.3 Determination of Foreign Matter**

Sample No.	Weight of sample		After removing impurities		% of foreign matters		Std. value
	Cultivated	Wild	Cultivated	Wild	Cultivated	Wild	
1	47.38	40.67	47.29	40.49	0.18	0.44	Not more than 2%
2	50	40	49.86	39.94	0.28	0.15	
3	50	40	49.89	39.88	0.22	0.3	

Both of the sample lies within the parameters as mentioned in reference standard.

### 8.4.2 Determination of Total Ash

**Table 8.4 Determination of Total Ash**

Sr. No.	Total Ash (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	5.10	5.5	4.8	4.26	Not more than 8%
2	5.1	3.3			
3	4.2	4			

Both the sample lies within the limit as mentioned in reference standard.

### 8.4.3 Determination of Acid Insoluble Ash

**Table 8.5 Determination Acid Insoluble Ash**

Sr. No.	Acid Insoluble Ash (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	0.94	1.5	0.84	0.82	Not more than 1%
2	0.78	0.47			
3	0.82	0.5			

Both of the sample lies within the limit as reference standard.

#### 8.4.4 Determination of Water Soluble Ash

**Table 8.6: Determination of Water Soluble Ash**

Sr. No.	Water soluble Ash (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	3.39	4	3.77	3.45	NA
2	3.80	2.85			
3	4.14	3.5			

The value of water soluble ash is not given in the standard but the result reveals very less difference in the water soluble extractive value of wild and cultivated samples of *Rauwolfia serpentina*.

#### 8.4.5 Determination of Alcohol Soluble Extractive Value

**Table 8.7: Determination Alcohol Soluble Extractive Value**

Sr. No.	Alcohol Soluble Extractive Value (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	25.26	6.4	16.27	6.4	Not less than 4%
2	10.98	6.8			
3	12.57	6			

Both of the sample lies within the limit as mentioned in reference standard.

#### 8.4.6 Determination of Water Soluble Extractive Value

**8.8: Determination of Water Soluble Extractive Value**

Sr. No.	Water Soluble Extractive Value (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	11.46	10.4	11.57	11.1	Not less than 10%
2	11.68	12.8			
3	11.00	10			

Both of the sample lies within the limit as reference standard.

#### 8.4.7 Determination of Ether Soluble Extractive Value

##### 8.9: Determination of Ether Soluble Extractive Value

Sr. No.	Ether Soluble Extractive Value (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	2.98	3.19	2.89	3.14	NA
2	3.0	3			
3	2.8	3.24			

The ether soluble extractive value is not given in the reference standard but the results revealed that less ether soluble content present in the both of the sample.

#### 8.4.8 Determination of Moisture Content (Loss on Drying)

##### 8.10: Determination of Moisture Content (Loss on Drying)

Sr. No.	Loss on drying (%)		Mean (%)		Standard Value
	Cultivated	Wild	Cultivated	Wild	
1	11.46	9.03	8.73	12.83	NA
2	11.15	11.4			
3	3.59	18.06			

The moisture content is not given in the standard but found to be more in wild variety as compare to the cultivated variety.

#### 8.5 Preliminary Phytochemical Investigations

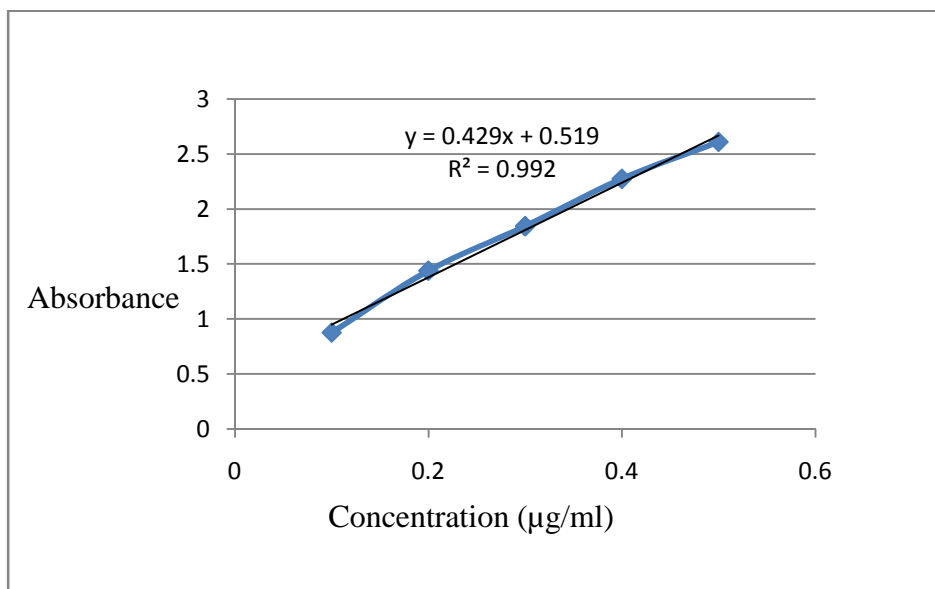
**Table 8.11: Phytochemical investigation of various extracts of *Rauwolfia serpentina* (wild & cultivated)**

S No	Test name		Extracts					
			Aqueous		Alcohol		Pet. Ether	
			Cultivated	Wild	Cultivated	Wild	Cultivated	Wild
1	Alkaloids	Mayer's reagent	+	+	+	+	+	+
		Dragondorrf's	+	+	+	+	+	+
		Hager's reagent	+	+	+	+	+	+

		Picrolinic acid	+	+	+	+	+	+
2	Tannins and phenolic compounds		+	+	+	+	+	+
		5% FeCl <sub>3</sub>	-	-	-	-	-	-
		Iodine	+	+	+	+	+	+
3	Glycosides	Baljet test	-	-	+	+	+	+
		Legal's test	+	+	+	+	-	-
		Test for Coumarin	+	+	+	+	+	+
4	Carbohydrates	Fehlings A and B	+	+	+	+	+	+
		Barfoerd's test	+	+	-	-	-	-
		Ninhydrine	+	+	+	+	+	+
5	Saponin		+	+	-	-	-	-
6	Volatile oil	Sudan III	-	-	-	-	-	-
7.	Steroids	Slowsky reaction	-	-	+	+	-	-
8.	Resins		+	+	-	-	-	-
9	Protein	Buired test	+	+	-	-	-	-

The aqueous extract of the plant *Rauwolfia serpentina* shows +ve results for alkaloids, tannins, carbohydrates, resins, saponins and proteins while the alcoholic extract shows +ve results for alkaloids, glycosides, steroids and comparatively less amount for tannic acid and carbohydrates. On the other hand, Pet. ether extract of the plant shows +ve results for alkaloids, and comparatively less for tannic acid, glycosides and carbohydrates are present in it.

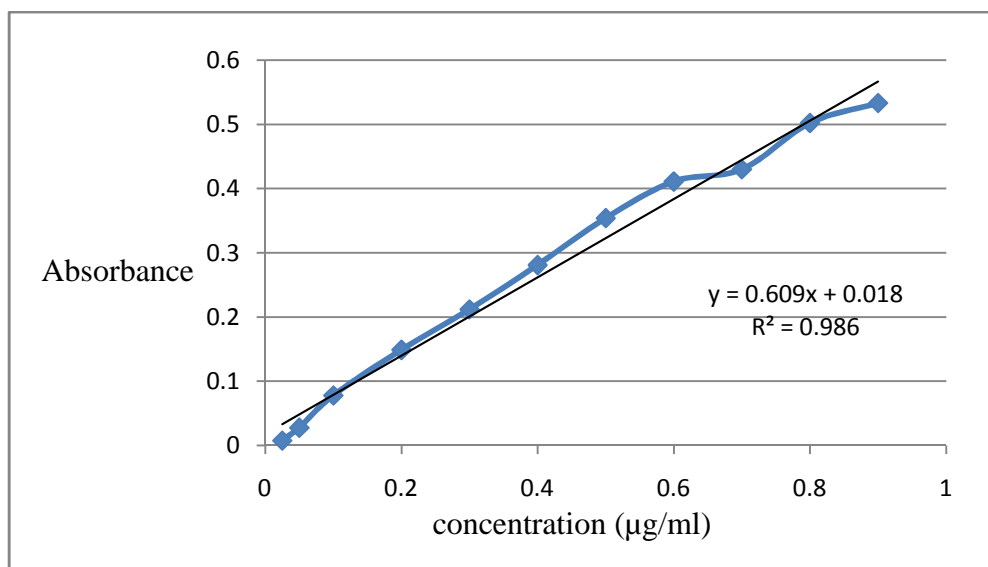
### 8.6 Estimation of Total Phenolic content (TPC)



**Figure 8.18:** Standard graph of Gallic acid

TPC is determined by the standard graph of gallic acid solution in different concentration as 0.1 ml, 0.2 ml, 0.3 ml, and it was found as 36.5, 79.0, 127.13 and 27.9, 37.8, 60.3 mg/ml in wild and cultivated sample of *Rauwolfia serpentina* respectively. The wild variety contains more amount of phenolic content.

### 8.7 Estimation of Total Tannic Acid content



**Figure 8.19:** Standard graph of tannic acid

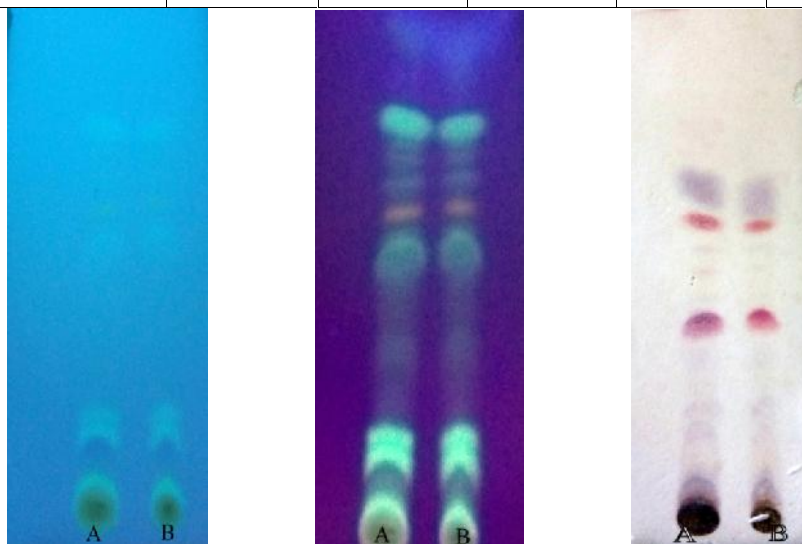
The tannic acid was determined by using the standard graph of tannic acid solution in different concentration as 0.1 ml, 0.2 ml, 0.25 ml and it was found as 24.5, 52.7, 58.7 and 12.9, 50.9, 52.5 mg/ml in wild and cultivated sample of *Rauwolfia serpentina* respectively.

### 8.8 Thin Layer Chromatography (TLC):

Various trails have been done for the separation of the constituents present in the cultivated and wild variety of *Rauwolfia serpentina*. The best separation was seen in solvent system using chloroform: methanol in the ratio (9.7:0.3) respectively. Alcoholic extract of the *Rauwolfia serpentina* shows best separation in this ratio.

**Table 8.12: Detail of TLC**

Extract	Mobile Phase (Ratio)	Rf			Rf		
		Wild			Cultivated		
		Day light	UV light	After spray	Day light	UV light	After spray
Methanolic	Chloroform : methanol (9.3:0.3)	0.07, 0.32, 0.61	0.07, 0.18, 0.32, 0.44, 0.55, 0.61	0.32, 0.55, 0.61	0.06, 0.32, 0.61	0.06, 0.18, 0.32, 0.44, 0.55, 0.61	0.32, 0.55, 0.61

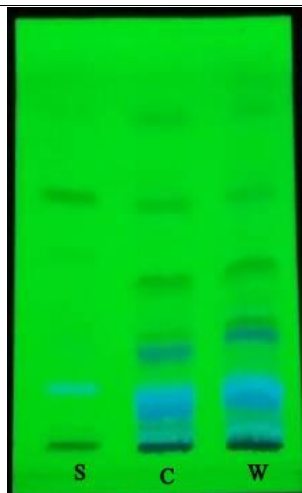


**Figure 8.20:** TLC of *Rauwolfia serpentina* plate 1 for day light, plate 2 for UV light and plate 3 after spraying respectively (A= Wild, B= Cultivated)



## 8.9 HPTLC

HPTLC study of the wild and cultivated extract of *Rauwolfia serpentina* with the standard Reserpine shows that there is the presence of Reserpine at around Rf 0.6

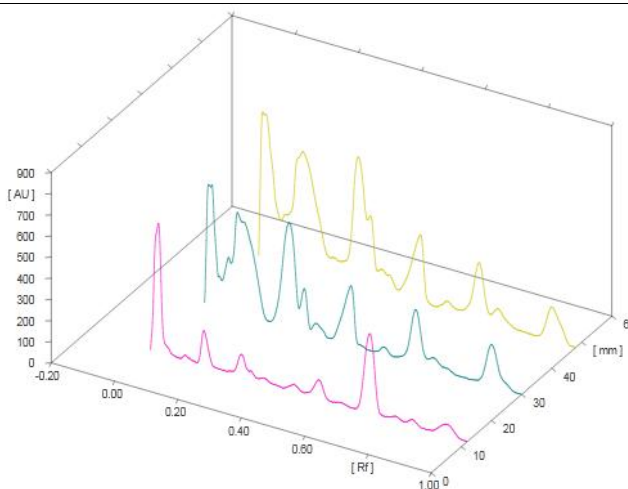


**Figure 8.21:** HPTLC of *Rauwolfia serpentina* with standard reserpine S= standard, C= cultivated, W= wild sample of plants



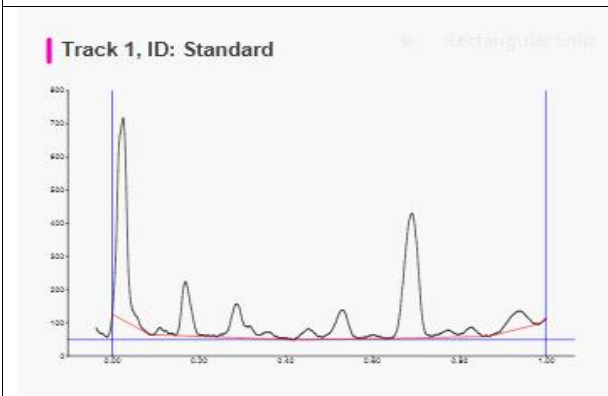
**Figure 8.22:** HPTLC of *Rauwolfia serpentina* with standard reserpine S= standard, C= cultivated, W= wild sample of plants

HPTLC study of the wild and cultivated extract of *Rauwolfia serpentina* with the standard Reserpine shows different colour bends which shows the presence of presence of reserpine in it.

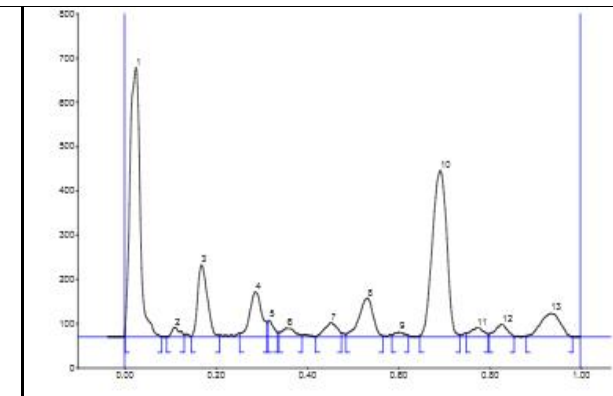


**Figure 8.23:** 3-D graph of standard, cultivated and wild *Rauwolfia serpentina*

### Standard drug Reserpine



**Figure 8.24: Graph for standard Reserpine**

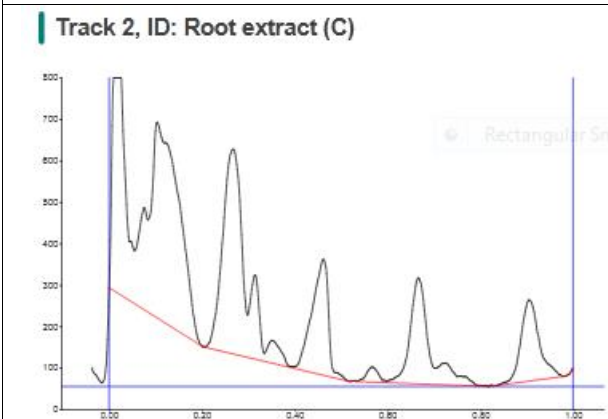


**Figure 8.25: Graph for standard Reserpine**

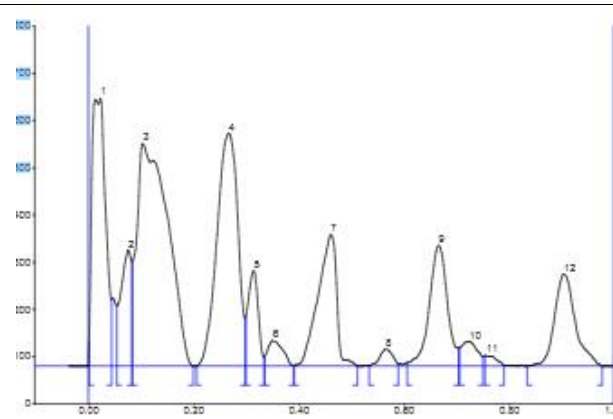
**Table 8.13: Interpretation table for standard Reserpine**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	20.3	0.02	608.8	38.89	0.08	1.7	12063.1	33.18
2	0.09	0.3	0.11	21.8	1.39	0.13	5.9	350.4	0.96
3	0.15	1.3	0.17	163.1	10.42	0.21	4.0	3105.0	8.54
4	0.25	8.7	0.29	102.2	6.53	0.31	33.7	2427.8	6.68
5	0.31	33.9	0.32	37.9	2.42	0.34	10.5	449.8	1.24
6	0.34	10.6	0.36	20.0	1.28	0.39	4.6	533.7	1.47
7	0.42	0.3	0.45	33.3	2.12	0.48	8.9	817.2	2.25
8	0.48	6.8	0.53	87.6	5.59	0.57	2.6	2447.7	6.73
9	0.59	4.3	0.60	11.4	0.73	0.62	4.2	230.2	0.63
10	0.65	2.8	0.69	376.1	24.02	0.74	5.4	10599.8	29.15
11	0.75	8.6	0.77	21.2	1.35	0.80	7.0	543.5	1.49
12	0.80	7.1	0.83	28.5	1.82	0.85	0.6	689.4	1.90
13	0.88	2.5	0.94	53.8	3.44	0.98	0.1	2099.1	5.77

Peak	Start Rf	Area
10.	0.65	10599.8



**Figure 8.26: Graph for cultivated root extract**

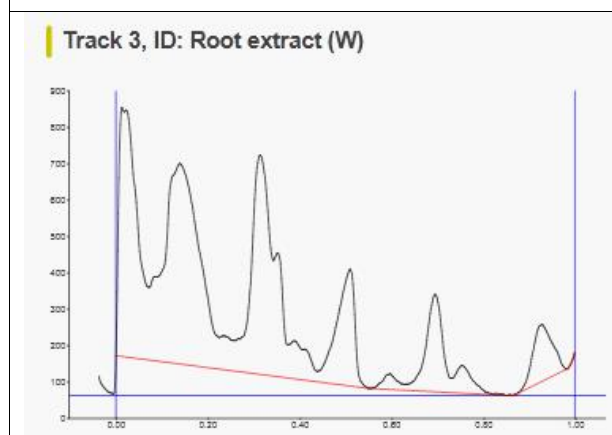


**Figure 8.27: Graph for cultivated root extract**

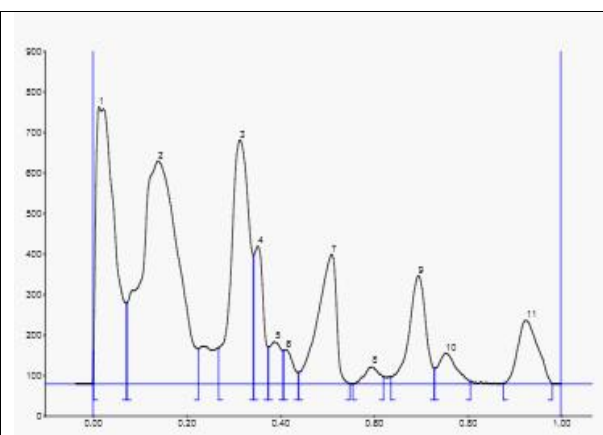
**Table 8.14: Interpretation table for cultivated root extract**

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	51.8	0.02	567.4	19.71	0.04	141.4	13237.0	14.49
2	0.05	127.9	0.07	246.2	8.55	0.08	222.5	4510.1	4.94
3	0.08	222.8	0.10	472.0	16.40	0.20	0.1	24903.7	27.26
4	0.21	1.0	0.27	493.5	17.15	0.30	101.5	17495.7	19.15
5	0.30	104.9	0.31	202.4	7.03	0.33	19.4	3533.1	3.87
6	0.34	20.7	0.35	54.4	1.89	0.39	1.9	1412.9	1.55
7	0.39	2.1	0.46	279.3	9.70	0.51	1.0	8799.7	9.63
8	0.53	1.4	0.56	36.4	1.26	0.59	5.5	810.2	0.89
9	0.61	7.6	0.67	255.8	8.89	0.70	38.8	7867.5	8.61
10	0.71	40.3	0.72	52.8	1.83	0.75	20.8	1446.6	1.58
11	0.75	22.1	0.76	22.5	0.78	0.79	2.4	448.5	0.49
12	0.84	0.1	0.91	195.4	6.79	0.98	0.0	6901.4	7.55

Peak	Start Rf	Area
3	0.08	24903.7
4	0.21	17495.7
7	0.39	8799.7
9	0.61	7867.5
12	0.84	6901.4



**Figure 8.28: Graph for wild variety of *Rauwolfia serpentina***



**Figure 8.29: Graph for wild variety of *Rauwolfia serpentina***

**Table 8.15: Interpretation table for wild variety of *Rauwolfia serpentina***

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	81.2	0.01	684.5	21.18	0.07	199.6	24524.9	19.80
2	0.07	200.1	0.14	550.8	17.05	0.22	86.4	39153.8	31.60
3	0.27	89.1	0.31	602.7	18.65	0.34	317.9	20705.0	16.71
4	0.34	318.4	0.35	340.4	10.53	0.37	90.8	5959.5	4.81
5	0.37	91.2	0.39	103.7	3.21	0.41	82.8	2382.0	1.92
6	0.41	83.1	0.41	85.5	2.64	0.44	28.4	1519.4	1.23
7	0.44	28.9	0.51	320.5	9.92	0.55	0.1	11310.4	9.13
8	0.55	0.4	0.60	42.8	1.33	0.62	17.7	1181.2	0.95
9	0.64	17.5	0.69	267.6	8.28	0.73	39.0	8527.0	6.88
10	0.73	39.0	0.75	76.3	2.36	0.81	6.3	2592.5	2.09
11	0.88	2.3	0.93	156.6	4.85	0.98	0.4	6030.5	4.87

Peak	Start Rf	Area
2	0.07	39153.8
3	0.27	20706.0
4	0.34	5959.5
7	0.44	11310.4
9	0.64	8527.0
11	0.88	6030.5

HPTLC study of the wild and cultivated extract of *Rauwolfia serpentina* with the standard Reserpine shows that different peaks are present in the extracts but the most prominent or the peak which resembles to the standard i.e. reserpine is around 0.6 Rf and the table also shows the

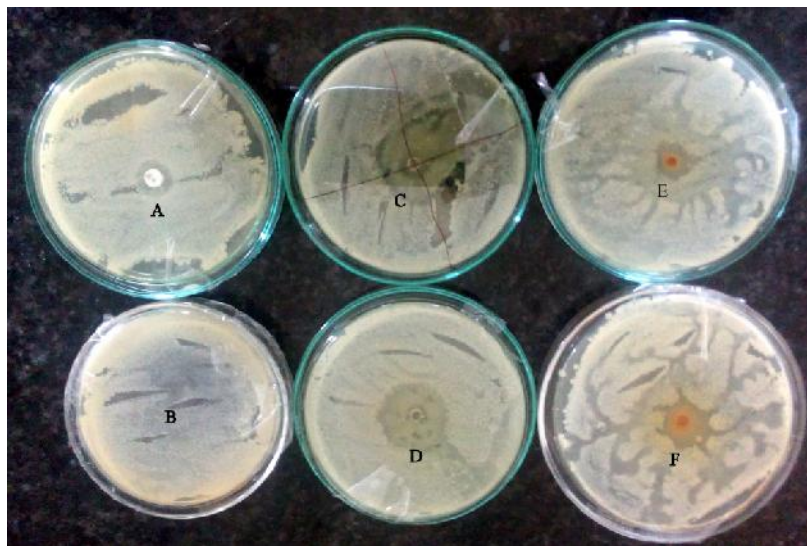
presence of other constituents present in the samples which can further be identified.

### 8.10 Antimicrobial study

**Table 8.16: Zone inhibition in mm for alcoholic and aqueous extracts of *Rauwolfia serpentina* on *E. coli***

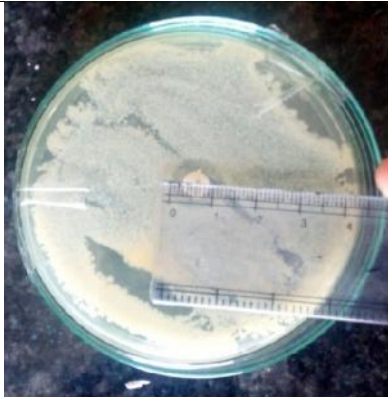
Sr. No.	Root extract	Control	Standard	Root extract	
				Wild	Cultivated
1.	Methanolic	0 mm	8 mm	12 mm	14 mm
2.	Aqueous			24 mm	25 mm

The aqueous extract of the roots of wild and cultivated plants of *Rauwolfia serpentina* shows the highest zone inhibition against the *Escherichia coli* in Mueller-Hinton agar culture media. According to the observations of 1 mg/ml concentration of wild and cultivated root methanolic extract shows 12 mm and 14 mm inhibition and the 1 mg/ml concentration of the aqueous extract of the wild and cultivates plant shows 24 mm and 25 mm respectively.



**Figure 8.30:** A = standard, B= control, C=alcoholic extract (wild), D=alcoholic extract (cultivated), E=aqueous extract (wild), F= aqueous extract (cultivated) for antimicrobial activity of the *Rauwolfia serpentina*

**Zone inhibition of the *Escherichia coli* by *Rauwolfia serpentina***



**Figure 8.31:** Standard drug (penicillin)

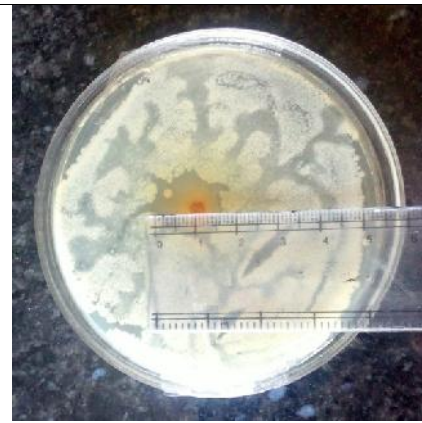


**Figure 8.32:** Control (water)

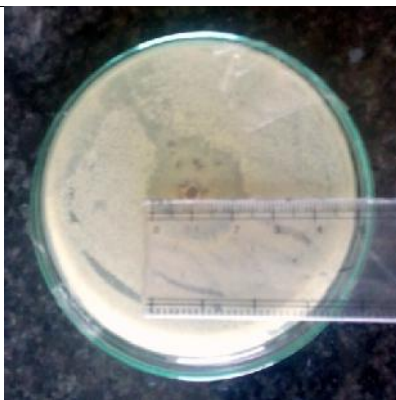
***Rauwolfia serpentina***



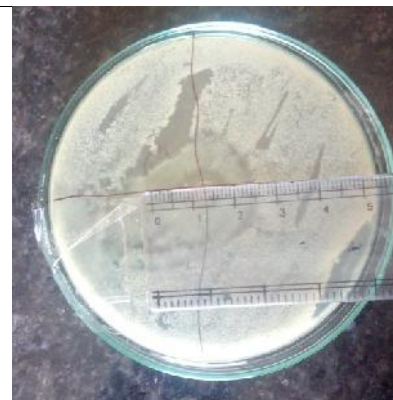
**Figure 8.33:** Wild alcoholic extract of plant for antimicrobial activity



**Figure 8.34:** Cultivated alcoholic extract for antimicrobial activity

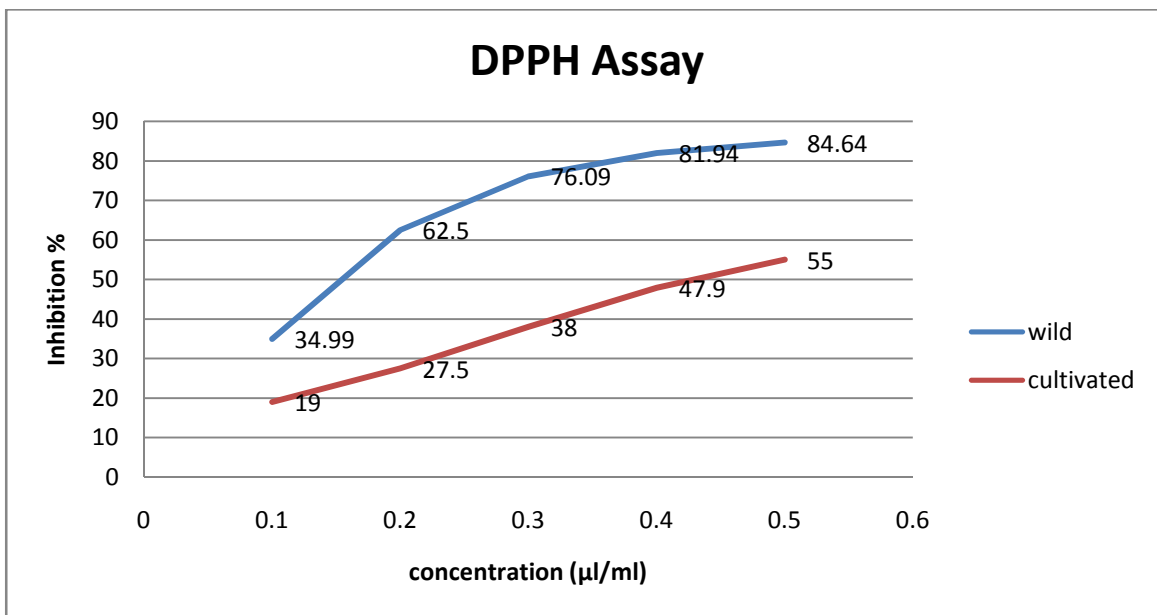


**Figure 8.35:** Wild aqueous extract for antimicrobial activity



**Figure 8.36:** Cultivated aqueous extract for antimicrobial activity

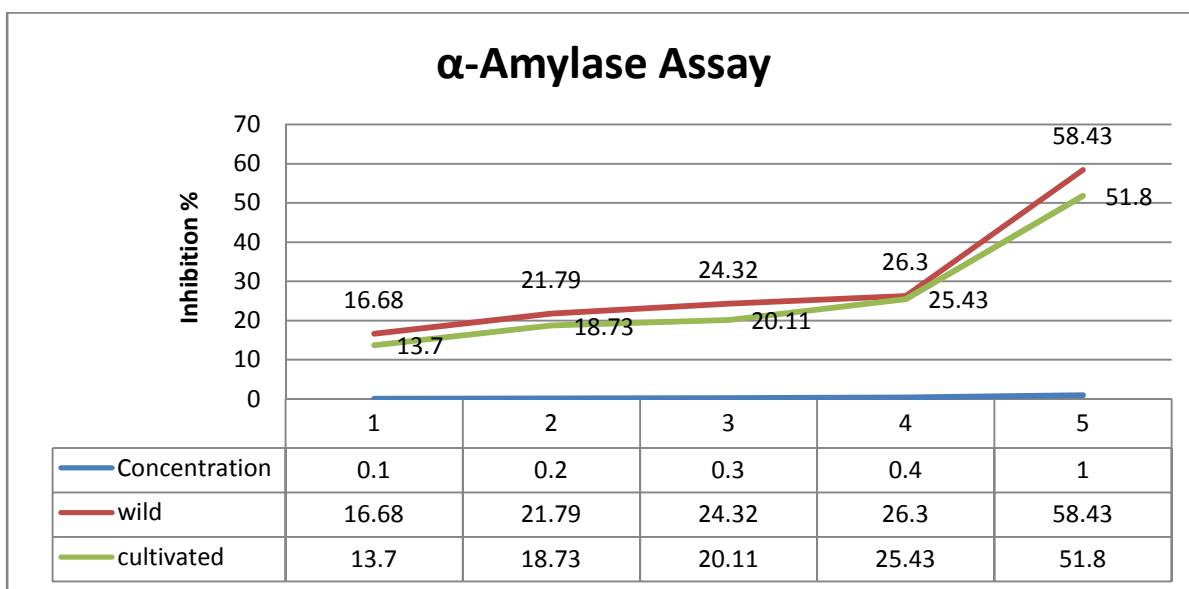
### 8.11 Antioxidant Activity Using DPPH Scavenging Assay



**Figure 8.37: Inhibitory %age of the wild and cultivated plant extracts**

The dose-dependent increase is found in percentage inhibition against DPPH Assay. At 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml concentration of extract showed a percentage inhibition of 19, 27.5, 38, 47.9 and 55 for cultivated and 34.99, 62.5, 76.09, 81.94 and 84.64 for wild respectively. The wild variety of the plant shows more inhibition as compare to the cultivated one.

### 8.12 Antidiabetic Activity using $\alpha$ -Amylase Inhibitory Assay



**Figure 8.38: Inhibitory %age of the wild and cultivated plant extracts**

Dose-dependent increase is seen in the percentage inhibition against  $\alpha$ -amylase enzyme activity. At 0.1, 0.2, 0.3, 0.4 and 1.0  $\mu\text{g/ml}$  concentration of extract showed a percentage inhibition of 13.7, 18.73, 20.11, 25.43 and 51.80 for cultivated and 16.68, 21.79, 24.32, 26.30 and 58.43 for wild respectively. The wild variety of the plant shows more inhibition as compare to the cultivated one.

## CHAPTER IX

### CONCLUSION AND FUTURE SCOPE

*Rauwolfia serpentina* L. Benth Kurz, is a antihypertensive drug mainly known for its phytochemical reserpine and is commonly called as Sarpagandha. It is widely described in classical text like Charak Samhita, Sushruta Samhita and Nighantus etc. In ancient time the plant is explained by various synonyms as Nakuli, Sarpagandha, Dhavalbaruaa, Harkai, Dhavalvipta, Nai, Chandra, Sanochado, Isargaj, vishhanti etc. and it is also renowned as “pagal ki buti” as it is used in mental illness.

The people rely on the plant since ancient time and used it blindly as it is effective in various major problems like insect poisoning, mental disorders, hypertension etc. And now a day’s 80% of the world population rely on herbal treatment. Due to the indiscriminate use of the plant it becomes endangered. So we have to find out some ways to prevent the plant from extinction.

The review includes the presence of the plant since antiquity. The plant posses the bitter (tikta) and pungent (katu) taste, dry (ruksha) and light (laghu) guna, hot (ushna) potency with sedative (nidrajanan) as specific action. It is reported to be kaphavatahar. Due to its ushna property it will help to pacify kapha and vata dosha. Due to vatashamak, it is used in mental disorders. Due to ushna veerya, it increased pitta and due to tikta rasa, it it used as krimighana, aampachan and jwaraghana.

This research work is done for comparing the wild and cultivated varieties of the plants in which various investigations are incorporated. The slight differences are observed in the macro and microscopic characters of the two plants. The physicochemical parameters are done for the wild and cultivated variety of *Rauwolfia serpentina* in which it is found that the alcohol soluble extractive value is more in case of cultivated variety as compare to wild variety and the other parameters are almost similar.

Then the preliminary phytochemical investigation shows the presence of alkaloids, carbohydrates, tannins, glycosides and fewer amounts of protein and steroids in both of the plants.

The qualitative and quantitative studies show the presence of tannins and phenolic content in which tannic acid content is found to be more in wild variety as compare to the cultivated one. TLC & HPTLC studies are also done in the wild and cultivated plants of *Rauwolfia serpentina* with the standard Reserpine and it was found that reserpine is present in both the samples at



about Rf 0.6.

Then the sample is taken for in-vitro antimicrobial, antioxidant and antidiabetic studies. The microbial and - amylase inhibition is moreover similar but in case of antioxidant activity the wild source of the plant have more potential to exert antioxidant activity as compare to the cultivated variety.

As the activity is reported in cultivated variety also, so if wild variety not available, then cultivated variety can be taken as substitute.

Future scope: DNA fingerprinting and in-vivo studies are required to further explore the therapeutic efficacy of wild and cultivated variety of *Rauwolfia serpentina*.

## CHAPTER X

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# CHAPTER XI

## APPENDIXES



**LOVELY  
PROFESSIONAL  
UNIVERSITY**  
*Transforming Education, Transforming India*

**TOPIC APPROVAL PERFORMA**

LIT (Pharmacy)/Department of Pharmaceutical Sciences

Program : P570-NN7::M.Pharm. (Ayurveda)

COURSE CODE : APH623

REGULAR/BACKLOG : Regular

GROUP NUMBER : PHRRGD0033

Supervisor Name : Amrinder Kaur

UID : 11662

Designation : Assistant Professor

Qualification : \_\_\_\_\_ Research Experience : \_\_\_\_\_

SR.NO.	NAME OF STUDENT	REGISTRATION NO	BATCH	SECTION	CONTACT NUMBER
1	Saveena Chauhan	11507125	2015	Y1553	8679838882

SPECIALIZATION AREA : Ayurvedic Pharmacy

Supervisor Signature: \_\_\_\_\_

PROPOSED TOPIC : Comparative pharmacognostic and phytochemical evaluation of cultivated and wild variety of *Rauwolfia serpentina* (Apocynaceae)

Qualitative Assessment of Proposed Topic by PAC		
Sr.No.	Parameter	Rating (out of 10)
1	Project Novelty: Potential of the project to create new knowledge	5.80
2	Project Feasibility: Project can be timely carried out in-house with low-cost and available resources in the University by the students.	7.00
3	Project Academic Inputs: Project topic is relevant and makes extensive use of academic inputs in UG program and serves as a culminating effort for core study area of the degree program.	7.00
4	Project Supervision: Project supervisor's is technically competent to guide students, resolve any issues, and impart necessary skills.	7.40
5	Social Applicability: Project work intends to solve a practical problem.	6.80
6	Future Scope: Project has potential to become basis of future research work, publication or patent.	6.60

PAC Committee Members		
PAC Member 1 Name: Dr. Amit Mittal	UID: 13145	Recommended (Y/N): Yes
PAC Member 2 Name: Saurabh Singh	UID: 12208	Recommended (Y/N): Yes
PAC Member 3 Name: Dr. S. Tamilvanan	UID: 16391	Recommended (Y/N): Yes
PAC Member 4 Name: Dr. Navneet Khurana	UID: 18252	Recommended (Y/N): Yes
DAA Nominee Name: Dr. Sazal Patyar	UID: 17050	Recommended (Y/N): Yes

**Final Topic Approved by PAC:** Comparative pharmacognostic and phytochemical evaluation of cultivated and wild variety of *Rauwolfia serpentina* (Apocynaceae)

**Overall Remarks:** Approved

PAC CHAIRPERSON Name: 11045::Dr. Monica Gulati

Approval Date: 27 Apr 2017

4/27/2017 10:36:04 AM

T. R.—5,  
GOVERNMENT OF HIMACHAL PRADESH

Dated 23/5/16

N<sup>o</sup> 4255470 B

Received from *Ms. Savina Chahal*  
*W.O. Paderi Mandi*  
the sum of Rs. *ONE HUNDRED FIFTY*  
in payment of *Medicinal Plant*  
Rs. *1.457*...

Cashier. Botanist  
Research Institute in ISM *Head of Office.*  
P&SHPS—4453-CP 28/99-29-31-2002-30,000 Books.  
Joginder Nagar, Distt. Mandi, 171





ਬੋਟੈਨੀਕਲ ਐਂਡ ਐਨਵਾਇਰਨਮੈਂਟਲ ਸਾਇੰਸਿਜ਼ ਵਿਭਾਗ

ਗੁਰੂ ਨਾਨਕ ਦੇਵ ਯੂਨੀਵਰਸਿਟੀ, ਅੰਮ੍ਰਿਤਸਰ - 143 005

Department of Botanical & Environmental Sciences  
Guru Nanak Dev University, Amritsar - 143 005, India

(Established by the State Legislature Act No. 21 of 1969)

Accredited at "A" grade level by NAAC and awarded "University with Potential for Excellence" status by UGC

Ref. No. 1335 Bot. & Env. Sc.

Dated 24-11-2016

To Whom It May Concern

The plant specimen(s) brought by Ms. Saveena Chauhan  
student of M. Pharmacy, Regn. No. 11507125,  
L.P.U. Phagwara belongs to the following species.

1. Rauwolfia serpentina (Sarpagandha)
2. Apocynaceae.
- 3.

Signature of Student [Signature]

Herbarium Assistant [Signature]

Teachers Incharge [Signature]

[Signature]  
Head  
Dept. of Botanical &  
Environmental Sciences  
Guru Nanak Dev University  
Amritsar-143005.

Phone: +91-183-2451048, PABX: 0183-2258802-09, 2450601-14 Extn. 3193, Fax : 0183-2258819-20 and 2255711  
Website: <http://www.gndu-dobes.org>; e-mail: [gndu\\_botanical@hotmail.com](mailto:gndu_botanical@hotmail.com)

**Retail Invoice (Form VAT XIX)**

(Original)

**Research Aid Instruments & Services**  
 Prabhu Chhaya Bhawan, Baijnath Road  
 Palampur-176061 (HP)  
 01894-231075, 231808 Telefax  
 Regd BO: Baddi (Rabaddi@gmail.Com)  
 E-Mail : researchaidpip@gmail.com

**Buyer**  
**Saveena Chauhan**  
 Lovely Professional University  
 Phagwara-144401

Invoice No. <b>C-54111</b>	Dated <b>19-Apr-2017</b>
Delivery Note	Mode/Terms of Payment
Supplier's Ref.	Other Reference(s)
Buyer's Order No.	Dated <b>19-Apr-2017</b>
Email	Delivery Note Date
Despatch Document No.	
Despatched through	Destination
Terms of Delivery	

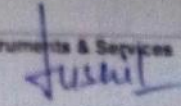
Sl No	Description of Goods	Quantity	Rate	per	Disc. %	Amount
1	<b>L350603 Reserpine 99% 1g</b>	<b>1 Pack</b>	4,813.00	Pack		<b>4,813.00</b>
	<b>CST Sale @13.75%</b>				13.75 %	<b>661.79</b>
	<b>Round Off +/-</b>					<b>0.21</b>
<b>Total</b>		<b>1 Pack</b>				<b>₹ 5,475.00</b>

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**Indian Rupees Five Thousand Four Hundred Seventy Five Only**

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 Baijnath Road, Palampur-176061 (HP)  
 Tel : 01894-231075, 231808

Company's VAT TIN : 02060700630  
 Company's CST No. : 02060700630  
 Company's PAN : AACFR9306A

Declaration  
 Input VAT Credit is not available against this invoice

for Research Aid Instruments & Services  
  
 Authorised Signatory

SUBJECT TO PALAMPUR JURISDICTION  
 This is a Computer Generated Invoice

winCATS Planar Chromatography Manager

Herbal Health Research Consortium  
Amritsar 143 001  
Punjab

**Analysis Report**

SOP document  
Validated  
Description : Design  
Analysis  
Created/used by E:\DATA\Rauwolfia serpentina 260417.cna  
Current user Admin Wednesday, April 26, 2017 1:42:32 PM  
Admin

**Stationary phase**

Executed by Admin Wednesday, April 26, 2017 11:59:31 AM  
Plate size (X x Y) 6.0 x 10.0 cm  
Material  
Manufacturer  
Batch  
GLP code  
Pre-washing  
Modification No  
No

**Definitions - Quantification**

Executed by Admin Wednesday, April 26, 2017 11:59:32 AM

**Calibration parameters**

Calibration mode Single level  
Statistics mode CV  
Evaluation mode Peak Height & Area

**Samples**

Sample ID: Standard  
Sample ID: Root extract (C)  
Sample ID: Root extract (W)

**Sample application - CAMAG Linomat 5**

Instrument CAMAG Linomat 5 "Linomat5\_180745" S/N 180745 (1.00.12)  
Executed by Admin Wednesday, April 26, 2017 12:12:02 PM

**Linomat 5 application parameters**

Spray gas : Inert gas  
Sample solvent type : Methanol  
Dosage speed : 150 nl/s  
Predosage volume : 0.2 ul

**Sequence**

Syringe size: 100 µl  
Number of tracks: 3  
Application position Y : 8.0 mm  
Band length : 10.0 mm

No.	Appl. position	Appl. volume	Vial #	Sample ID	Active
>1	12.0 mm	15.0 µl	1	Standard	Yes
>2	30.0 mm	15.0 µl	2	Root extract (C)	Yes
>3	48.0 mm	15.0 µl	3	Root extract (W)	Yes

User: Admin  
Wednesday, April 26, 2017 1:42:33 PM

Approved : .....  
Report ID : 07E1041A040D2A20

SN 1809W062, V1.4.6  
Page 1 of 5

winCATS Planar Chromatography Manager

Detection - CAMAG TLC Scanner

**Information**

Application position 8.0 mm  
Solvent front position 85.0 mm

**Instrument**

Executed by CAMAG TLC Scanner "Scanner\_180710" S/N 180710 (2.01.02)  
Number of tracks Admin Wednesday, April 26, 2017 1:40:33 PM  
Position of first track X 3  
Distance between tracks 12.0 mm  
Scan start pos. Y 18.0 mm  
Scan end pos. Y 5.0 mm  
Slit dimensions 85.0 mm  
Optimize optical system 4.00 x 0.30 mm, Micro  
Scanning speed: Light  
Data resolution: 20 mm/s  
100 µm/step

**Measurement Table**

Wavelength 254  
Lamp D2 & W  
Measurement Type Remission  
Measurement Mode Absorption  
Optical filter Second order  
Detector mode Automatic  
PM high voltage 303 V

**Detector properties**

Y-position for 0 adjust 5.0 mm  
Track # for 0 adjust 0  
Analog Offset 10%  
Sensitivity Automatic (37)

**Integration**

**Properties**

Data filtering Savitsky-Golay 7  
Baseline correction Lowest Slope  
Peak threshold min. slope 5  
Peak threshold min. height 10 AU  
Peak threshold min. area 50  
Peak threshold max. height 990 AU  
Track start position 8.0 mm  
Track end position 85.0 mm  
Display scaling Automatic

User : Admin  
Wednesday, April 26, 2017 1:42:33 PM

Approved : .....  
Report ID : 07E1041A040D2A20

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Page 2 of 5

# MADHU SAMVAADA

**Symposium on Developing Protocol for Management  
of Diabetes and its Complications**  
(17<sup>th</sup> & 18<sup>th</sup> March 2017)



**Abstract Book**

**ALL INDIA INSTITUTE OF AYURVEDA**  
(An Autonomous Organisation Under the Ministry of AYUSH, Govt of India)  
New Delhi - 110076

## Validation and Comparison of Antidiabetic Activity of Wild and Cultivated plant of *Sarpagandha*

<sup>1</sup>Saveena Chauhan, Amrinder Kaur, Manish Vyas

1. M Pharma (Ayu) Scholar, School of Pharmaceutical Sciences, Lovely Professional University, Phagwara (saveena.chauhan@gmail.com)

**Introduction:** India is renowned as diabetes capital of the world because 50 million cases are diagnosed in 2016. It is a metabolic disorder caused due to high blood glucose levels as a consequence of inadequate secretion of insulin. Ayurvedic drugs have a huge contribution in the treatment of endocrine disturbance and diabetes by improving insulin sensitivity, production and decreasing blood glucose level. More than 80% of global population is reliant on natural resources due to their safety and efficacy, and over 800 plant species have been mentioned in literature with significant hypoglycemic activity. Among all these species *Sarpagandha* (*Rauwolfia serpentina*) is selected to validate its hypoglycemic activity. **Objective:** The objective of the study is to validate and compare the hypoglycemic activity of the wild and cultivated varieties of *Sarpagandha*. **Material & Methods:** The aqueous extracts of wild and cultivated plant were subjected to the  $\alpha$ -amylase inhibition activity for anti-diabetic study. **Result and Discussion:** The result explores an alternate for the endangered wild plant of *Sarpagandha*. However, wild plant is more effective for the management of diabetes as compare to cultivated plant.

**Keywords:** *Sarpagandha*, Antidiabetic activity, Hypoglycemia, *Rauwolfia serpentina*.

\*\*\*\*\*



# ALL INDIA INSTITUTE OF AYURVEDA

(An Autonomous Organization under the Ministry of AYUSH, Govt. of India)  
New Delhi



## MADHU SAMVAADA

Developing Protocol for Management of Diabetes and its complications

(15<sup>th</sup> to 18<sup>th</sup> March 2017)

### CERTIFICATE

This is to certify that Dr. Saveena Chauhan  
has contributed in the successful conduction of the Pre-symposium (15<sup>th</sup> and 16<sup>th</sup> March 2017) / Symposium (17<sup>th</sup> and 18<sup>th</sup> March 2017) organized by All India Institute of Ayurveda, New Delhi and contributed as

- Chairman  Co-Chairman  Rapporteur  Key Note Speaker
- Resource Person  Delegate  Presented a paper / poster entitled

*Validation & comparison of antidiabetic activity of wild & cultivated plant of Sarpa Gandha.*

*Dr. Galib*  
Organizing Secretary

Prof. P K Prajapati  
Co-Chairman

*Prof. Abhimanyu Kumar*  
Chairman

Prof. Abhimanyu Kumar  
Chairman



ORIGINALITY REPORT

% **10**

SIMILARITY INDEX

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INTERNET SOURCES

% **7**

PUBLICATIONS

% **5**

STUDENT PAPERS

PRIMARY SOURCES

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<b>3</b>	Submitted to Higher Education Commission Pakistan Student Paper	% <b>1</b>
<b>4</b>	www.ukessays.com Internet Source	% <b>1</b>
<b>5</b>	www.ibdr.in Internet Source	<% <b>1</b>
<b>6</b>	Edible Medicinal And Non-Medicinal Plants, 2014. Publication	<% <b>1</b>
<b>7</b>	apjtb.com Internet Source	<% <b>1</b>
<b>8</b>	Saklani, Sarla; Mishra, Abhay P.; Parcha, Varsha and Chandra, Subhash. "Phytochemical	<% <b>1</b>