Pharmacognostical Study of Rauwolfia serpentine (Sarpagandha) Root

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INTRODUCTION
Ayurveda is a Sanskrit term, made up of the words “Ayus” and “veda”. Ayus means life and “veda” means knowledge or science. The term “Ayurveda” thus means ‘The Knowledge of Life’ or ‘The Science of Life’. According to the ancient Ayurvedic scholar Charaka, “Ayu” comprises the mind, body, senses and the soul. “Ayurveda is a science in the sense that it is a complete system. It is a qualitative, holistic science of health and longevity, a philosophy and system of healing the whole person, body and mind”. (Lad, Dr Vasant)

The aim of this system is to prevent illness, heal the sick and preserve life. This can be summed up as follow-
- To protect health and prolong life (“Swasthya swasthyarakshanam”)
- To eliminate diseases and disfunction of the body (“Aturasyavikarpashamanamcha”)

Herbal medicine are also called botanical medicine or phytomedicine which refers to use a plant’s seeds, berries, roots, leaves, bark, or flower. In many cases, scientists are not sure what specific ingredient in a particular herb works to treat a condition or illness. Whole herbs contain many ingredients, and they may work together to produce a beneficial effect. Many factors determine how effective an herb will be. For example, the type of environment (climates, bugs, soil quality) in which a plant grew will effect it, as will how and when it has harvested and processed.

The use of herbal supplement has increased dramatically over the past 30 years. Herbal supplements are classified as dietary supplement by the U.S Dietary Supplement Health and Education Act (DSHEA) of 1994, that means herbal supplements unlike prescription drugs can be sold without being tested to prove they are safe and effective. However, herbal supplements must be made according to good manufacturing practices.

The Ayurvedic drug are very useful for mankind so before using chemical and allopathic drug we should go for the natural and Ayurvedic drug. Sarpagandha is very good and useful drug. Sarpagandha is very useful for many diseases which is given below.

ABSTRACT
Rauwolfia serpentina is a medicinally famous herb in Ayurvedic and western system of medicine. Reserpine is an alkaloid and is important constituent of Rauwolfia serpentina which was reported to possess antibacterial activity. In the present study High Performance Thin Layer Chromatography was developed for detection monitoring and quantification of reserpine in Rauwolfia serpentina and various pharmacognostical parameter of Rauwolfia serpentina were also established for their correct identification.

Keyword: Rauwolfia serpentina, High Performance Thin Layer Chromatography, Reserpine, Antibacterial activity.
- It controls high blood pressure and hyper tension.
- It act as a appetizer and good for digestion.
- It increases menstrual bleeding.
- It is very useful in psychic diseases.

**Brief description of Plant**

**Rauwolfia serpentina (API)**

Sarpagandha consist air dried root of Rauwolfia serpentina family apocynaceae under shrub widely distributed in India in the sub Himalayan tracks upto 1000m as well as in the lower ranges of the eastern and western ghats and the Andman island.(API)

**Synonyms(API)**

Sanskrit- Nakulichandrika

English- Rauwolfia root

Hindi- Chhotachand

Gujrat- Amelpondi

(API)

**Classification**

Kingdom-Plantae

Division-Magnoliophyta

Class- Magnoliopsida

Order- Gentianales

Family- Apocynaceae

Genus- Rauwolfia

Species- *R. serpentina*

**Macroscopic**

**Root-** Pieces of roots mostly about 8 to 15 cm. long and 0.5 to 2 cm in thickness sub cylindrical curved stout thick and rarely branched outer surface grayish yellow to brown with irregular longitudinal fissures rootlets 0.1 mm. India fracture short sling odour and bitter taste.(API)

**Microscopic**

**Root powder:**

Coarse to fine yellowish brown free flowing odour slight bitter in taste characterized by spherical simple to compound starch grains calcium oxalate prism and cluster vessels with simple perforation occasionally tailed trachieds lignified, xylem, fibers irregular in shape occurs singly or in small groups walls lignified tips occasionally wood parenchyma cells are filled with calcium oxalate crystals and starch grains stone cells ,phloem fibers absent.(API)

**Chemical constituents**

Indole, alkaloid, alkaloid, such as reserpine, serpentine and ajmalicine.(API)

**Part used-** root

**Pharmacology Action**

Therapeutic uses

1. Circulatory System-High blood pressure, hypertension.
2. Nervous system- The analgesic sedative are probably through the depletion of tissue stores catchotamines from peripheral sites and serotonin from the brain. Useful in migraine, neuropsychiatric insomnia.
3. Digestive System- It’s act as appetizer and good for digestion. Digestive use in dysentery and painful bowel disorder, stops vomiting.
4. Reproductive System- It increase menstrual bleeding. It is also use to increase uterine contraction. (QUALITY STANDARD)

**Identity, Purity and Strength**

Foreign matter- Not more than 2%
Total ash - Not more than 8%
Acid insoluble -Not more than 1%
Alcohol soluble extractive - Not less than 4%
Water soluble extractive - Not less than 10%

(API)

**MATERIAL AND METHOD**

**Plant materials**

*Rauwolfia serpentina* (root) was collected from Deendayal Research institute and other places, Chitrakoot in February-March 2014. They were washed with tap water, rinsed with distilled water and shade dried until the fracture is uniform and smooth. The dried root material was powdered and was used for analysis.

The analysis was carried in following heads following the standard method of analysis.

**MICROSCOPY: (Anonymous API 2006)**

Powder microscopy was done in order to identify the dry powder and to determine which plant part was used as the drug. For the purpose, a very small amount of drug was placed over the clean, dry slide and two drop of glycerine was added and mixed. Then it was covered with cover slip and observed under the microscope after drying.

**Fluorescence test (Anonymous Basic guide lines)**

The fluorescence test were performed by using ana 1N HCl, 1N NaOH, 1N NaOH & Methanol, 50 % KOH, 50% H₂SO₄, Conc. H₂SO₄, 50% HNO₃, CH₃COOH, distilled water on different wavelength

1) A small amount of powdered drug was placed on a micro slide and observed under UV 366 and UV 254 nm.
2) A small amount of powdered drug was placed on a micro slide and treated with 1N HCl and observed under UV 254 and UV 366nm.
3) A small amount of powdered drug was placed on a micro slide and treated with 1N NaOH and observed under UV 254 and UV 366nm.
4) A small amount of powdered drug was placed on a micro slide and treated with 1N NaOH in methanol and observed under UV 254 and UV 366nm.
5) A small amount of powdered drug was placed on a micro slide and treated with 50% KOH and observed under UV 254 and UV 366nm.
6) A small amount of powdered drug was placed on a micro slide and treated with 50% H₂SO₄ and observed under UV 254 and UV 366nm.
7) A small amount of powdered drug was placed on a micro slide and treated with Conc. H₂SO₄ and observed under UV 254 and UV 366nm.
8) A small amount of powdered drug was placed on a micro slide and treated with 50% HNO₃ and observed under UV 254 and UV 366nm.
9) A small amount of powdered drug was placed on a micro slide and treated with acetic acid and observed under UV 254 and UV 366nm.
10) A small amount of powdered drug was placed on a micro slide and treated with wastewater and observed under UV 254 and UV 366nm.

**PHYSIOCHEMICAL EVALUATION OF THE DRUG: (Anonymous API 2006)**

The following parameter were determined
1. pH
2. LOD
3. Total ASH
4. Acid insoluble ash
5. Water insoluble ash
6. Extractive value

**pH value**: The pH value of an aqueous liquid may be defined as “the common logarithm of the
reciprocal of the hydrogen ion concentration expressed in gram per litre.

**Procedure:** Standardize the pH meter and electrodes with distilled water then buffer solution (Ph 9.0) and 0.05M potassium hydrogen phthalate (pH 4.0). After this take a reading of the sarpagandha sample. (Anonymous API 2006)

**Determination of Loss on Drying (LOD):**
Placed about 2 g of drug (without preliminary drying) after accurately weighed it in a evaporating dish. Then dried at 105 °C for 5 hrs and weighed. Repeat the same process two more times and weighing at 30 minutes an calculated by using the formula. (Anonymous API 2006)

\[
\text{Moisture content} = \frac{\text{Average value} \times 100}{\text{weight of sample taken}}
\]

**Determination of Total Ash:**
2g of air dried crude drug was taken a crucible and heated at temperature not exceeding 450°C. Then it was cooled in desiccators and weighed. The percentage of total ash was calculated with reference to air dried drugs. (Anonymous API 2006)

\[
\text{Total Ash} = \frac{\text{avg X 100}}{\text{Wt. of sample taken}}
\]

**Determination of Acid Insoluble Ash:**
The total ash obtained as mentioned in the above procedure was boiled with 25 ml of HCL for 5 min and filtered the insoluble matter was collected in an ash less filter paper, washed with hot water until the filtrate is neutral. Then the filter paper containing the insoluble matter was dried, transferred to crucible and ignited to constant weight, and then the residue was cooled in desiccators for 30 min and weighed. The percentage of acid insoluble ash was calculated by the following formula. (Anonymous API 2006)

\[
\text{Acid insoluble ash} = \frac{\text{avg X 100}}{\text{Wt. of sample taken}}
\]

**Determination of Water Insoluble Ash:**
The total ash obtained as mentioned in the above procedure was boiled with 25 ml of water for 5 min; the insoluble matter was collected in an ash less filter paper, washed with hot water until the filtrate is neutral. Then the filter paper containing the insoluble matter was dried, transferred to crucible and ignited to constant weight, and then the residue was cooled in desiccators for 30 min and weighed. The percentage of acid insoluble ash was calculated by the following formula. (Anonymous API 2006)

\[
\text{Water insoluble ash} = \frac{\text{avg X 100}}{\text{Wt. of sample taken}}
\]

**Extractive value:**

**Determination of ethyl alcohol soluble extractive:**
5 g of the air dried drug was macerated with 100 ml of ethyl alcohol and kept in a closed flask for 24 hours anshaked the contents on shaker for six hours then kept the contents for 18 hours and then followed evaporated 25 ml of the filtrate in a Petridish at 105 °C residue was weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drugs. (Anonymous API 2006)

\[
\text{Alcohol Soluble Extractive} = \frac{\text{avg value X 500}}{\text{Wt. of sample taken}}
\]

**Determination of Water soluble extractive:**
5 g of the air dried drug was macerated with 100 ml of distilled water and kept in a closed flask for 24 hours anshaked the contents on shaker for six hours then kept the contents for 18 hours and then followed evaporated 25 ml of the filtrate in a Petridish at 105 °C residue was weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drugs. (Anonymous API 2006)

\[
\text{Water Soluble Extractive} = \frac{\text{avg value X 500}}{\text{Wt. of sample taken}}
\]

**Determination of chloroform soluble extractive:**
5 g of the air dried drug was macerated with 100 ml of chloroform and kept in a closed flask for 24 hours anshaked the contents on shaker for six hours then kept the contents for 18 hours and then followed evaporated 25 ml of the filtrate in a Petridish at 105 °C residue was weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drugs. (Anonymous API 2006)

\[
\text{Chloroform Soluble Extractive} = \frac{\text{avg value X 500}}{\text{Wt. of sample taken}}
\]
Determination of methyl alcohol soluble extractive:
5 g of the air dried drug was macerated with 100 ml of methyl alcohol and kept in a closed flask for 24 hours anshaked the contents on shaker for six hours then kept the contents for 18 hours and then followed evaporated 25 ml of the filtrate in a Petridish at 105 °C residue was weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drugs.
(Anonymous API 2006)
Alcohol Soluble Extractive = avg value X 500

Phytochemical analysis: (Anonymous Basic guide line)
Different phytochemicals present in the plants were qualitatively analysed. The ethanol and aqueous extract were prepared and the different chemical compounds were detected.
Alkaloid- Alkaloids are a group of naturally occurring chemical compounds that contain mostly basic nitrogen atom. Alkaloids are produces by a large variety of organisms, including bacteria, fungi, Plant and Animals and are part of the group of natural product.
Wagner’s test for alkaloids: - 1 ml of alcoholic extract was acidified with HCl (v/v) and few drop of Wanger’s reagent was added to it. Presence of alkaloid is indicated by formation of yellow or brown coloured precipitate.
Carbohydrates- Carbohydrates are regarded as the poly hydroxyl aldehyde or poly hydroxyl ketone or substance that can be hydrolysed to either of them carbohydrate are produce in nature by green plant.
Fehling Test:- 2ml of aqueous extract is taken in a test tube and 1ml of a mixture of Fehling solution is added boiled for a few minutes, formation of brick red precipitates indicate presence of carbohydrates.
Flavonoid- Flavonoids are a class of plant secondary metabolites in higher plants flavonoids are involved in UV filtration symbiotic nitrogen fixation and floral pigmentation. They may acts as chemical messenger or physiological regulator; they can also act as cell cycle inhibition.
Test:- 5-10 drops of dil.HCl is added to 0.5ml of alcoholic extract of the drug and a piece of Mg metal was added, pink, reddish-pink or brown colour was developed.
Proteins- proteins are biochemical compounds consisting of one or more polypeptide typically folded into a globular form, facilitating a biological function, like other biological macromolecule such as polysaccharides and nucleic acid, protein are essential part of the organism and participate in virtually every process within cells.
Biuret test:- 1ml of hot aqueous extract, 5-8 drop of 10% NaOH solution was added followed by 1-2 drop of 3% copper sulphate. A violet colour indicated the presence of proteins.
Resin- Resin in the most specific use of the term is a hydrocarbon secretion of many plants. Particularly coniferous trees. Resin consist primarily of secondary metabolite or compound that apparently play no role in the primary physiology of plant while some scientist view resin only as waste product, their protective benefits to the plant are widely documented.
Test: - 1 ml of aqueous extract of the drug was dissolved in 2 ml of acetone and then solution is poured in a tube containing 2-3 ml of distilled water. Appearance of turbidity indicates the presence of Resin.
Saponins- Saponins are a class of chemical compounds. Test:- 5ml of aqueous extract, added a drop of sodium bicarbonate 1s added and shaken vigorously and left for few minutes, formation of honey comb like froth indicates the presence of saponin.
Steroid –
Test:- Added 1ml of conc. H2SO4 to 2ml of chloroform extract of drug from the side of test tube. A red colour is produced in the layer of chloroform indicates the presence of steroid.
Tannins- A tannin is astringing bitter plant polyphenolic compound that bind to and precipitate protein and various other organic compound including amino acid and alkaid. The Tannin compound are widely distributed in many species of plant, where they play a role in protection from predator and perhaps also from on pesticides and in plant growth regulator.
Test: - To 1-2 ml of aqueous extract, few drop of 5% ferric chloride was added. A green colour indicated the presence of allotannin where a brown colour Tannin.
Starch –starch are regarded as the poly hydroxyl aldehyde or poly hydroxyl ketone. 

Test:-dissolved 0.015 g of iodine and 0.075 g of KI in 5ml distilled water and added 2-3 ml aqueous extract of drug. Ablue colour was produced.

High Performance Liquid Chromatography.
(Anonymous Quality Standard 2010, Harish Kumar, Sangam Kumar)

High Performance Liquid Chromatography is a sophisticated and automated form of TLC techniques. This method is used for separation of the components presents in mixture both quantitatively as well qualitatively.

For quantitative analysis of medicinal plant sufficient quantity of ethanol extract after dissociating in methanol was passed through µ Millipore membrane filter unit. About 8µl was applied on High Performance Thin Layer Chromatography plates with a TLC sampler and was piloted by the WINCATS software 1.3.2 (CAMAG, Switzerland), chloroform dilution of a solution was prepared by dissolving .5g of drug in 25.0 ml chloroform and then applied on the plates. After development of plates in appropriate mobile phase solvent system (toluene: ethyl acetate: Diethylamine 7:2:1) the plates were scanned at particular wavelength in reflectance mode with a TLC scanner third (WINGATS 1.3.2, CAMAG).

Derivatization: In situ derivatization possibility is a strong point of High Performance Thin Layer Chromatography. Chemical reaction is possible in situ on the plate before or after chromatography both the possibilities have their advantages. However the decision depends on the sample matrix level of detection quantification and interference present. Post chromatography derivatization in more popular technique for which several hundred references in literature are available as compared to a few for pre-chromatographic deviation. The results are unique and specific when derivatization was after chromatographic development. In the study the derivatization was done after chromatographic development. (Anonymous Quality Standard 2010).

Preparation for High Performance Thin Layer Chromatography:

Test solution
.5g of coarsely powdered drug was mixed with 25 ml of chloroform in a conical flask then the content were filtered and concentrated.

Standard solution:
10 mg of reserpine was dissolved in a volumetric flask and volume was make up to 50 ml with chloroform then 1ml of the solution was taken in the flask it was made 10 ml with chloroform. (Anonymous Quality Standard 2010)

Stationary phase: The pre-coated plates with silica gel 60F 254 of 0.2 mm thickness.

Mobile phase:
toluene: ethylacetate: Diethylamine (7:2:1)

Spray reagent: Dragendorff reagent

Calibration curve:
Applied 1µl of reserpine standard solution in duplicates on the TLC plates. Developed the in the solvent system to a distance of 8 cm .Dried the plate in solvent system to obtained the chromatogram and determined the area of peak corresponding to that of reserpine as described above for the calibration curve by plotting peak area vs. concentration of reserpine applied.

Estimation of resepine in the drug:
Applied 1µl ofRauwolfia serpentina sample(root) solution in duplicates on the TLC plates. Developed the in the solvent system to a distance of 8 cm .Dried the plate in solvent system to obtained the chromatogram and determined the area of peak corresponding to that of reserpine as described above for the calibration curve. Calculated the amount of reserpine present in the sample from the calibration curve of reserpine. (Anonymous Quality Standard 2010).

Tannin estimation by U.V.Spectrophotometer
Extracted 2g powdered plant material with 100 ml distilled water on water bath filtered and volume was made up to 100 ml in volumetric flask. Took 1 ml aliquot of it and added 5ml of folin phenol reagent 10 ml saturated sodium carbonate and volume was made 100 ml and took the absorbance of the sample at 760nm. (Anonymous Basic guide line)
RESULT

Microscopy of Sarpagandha Root is given below

**TS: Diagrammatic**

- Cork
- Cortex
- Growth ring
- Xylem
- Phloem

**TS: Cork & Cortex**

- Starch grains
- Prismatic crystals of calcium oxalate
- Cork cambium
- Medullary rays
- Cork cells

**TS: Root**

- Latex cells
- Xylem vessels
- Starch grains

**Powder Microscopy of Sarpagandha Root**

- Prismatic crystals of calcium-oxalate
- Cork cells
- Medullary rays crossing the vessels
- Pitted vessels

**Pitted parenchymatous cells**
### Fluorescence test

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Reagent + powder</th>
<th>366 nm</th>
<th>254 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glycerine</td>
<td>Sky blue</td>
<td>Blue</td>
</tr>
<tr>
<td>2</td>
<td>1N hydrochloric acid</td>
<td>Sky blue</td>
<td>Navy blue</td>
</tr>
<tr>
<td>3</td>
<td>1N Sodium hydroxide</td>
<td>Fluorescence colour</td>
<td>Blue</td>
</tr>
<tr>
<td>4</td>
<td>1N Sodium hydroxide+methanol</td>
<td>Light green</td>
<td>Fluorescence colour</td>
</tr>
<tr>
<td>5</td>
<td>50% Potassium hydroxide</td>
<td>Fluorescence colour</td>
<td>Dark blue</td>
</tr>
<tr>
<td>6</td>
<td>50% Sulfuric acid</td>
<td>Sky blue</td>
<td>Blue</td>
</tr>
<tr>
<td>7</td>
<td>Conc. Sulfuric acid</td>
<td>Dark blue</td>
<td>Sky blue</td>
</tr>
<tr>
<td>8</td>
<td>50% Nitric acid</td>
<td>Sky blue</td>
<td>Sky blue</td>
</tr>
<tr>
<td>9</td>
<td>Acetic acid</td>
<td>Sky blue</td>
<td>Sky blue</td>
</tr>
<tr>
<td>10</td>
<td>Iodine water</td>
<td>Sky blue</td>
<td>Dark blue</td>
</tr>
</tbody>
</table>

#### Physicochemical parameter

**pH value of the drug:** 6.73

**LOD (Loss On Drying):**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Petri dish + powder</th>
<th>After 5 hour weight</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>18.40</td>
<td>18.27</td>
<td>17.94</td>
<td>0.15</td>
</tr>
<tr>
<td>2</td>
<td>18.06</td>
<td>17.94</td>
<td>17.91</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>16.33</td>
<td>16.27</td>
<td>16.18</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Average = 0.15**

**LOD = average X 100/weight of sample**

**LOD = 0.15X100/2 = 7.5%**

### Total ash

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Weight of crucible</th>
<th>1st day</th>
<th>2nd day</th>
<th>Final day</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>38.93</td>
<td>39.08</td>
<td>39.078</td>
<td>39.07</td>
<td>0.13</td>
</tr>
<tr>
<td>2</td>
<td>38.82</td>
<td>38.87</td>
<td>38.81</td>
<td>38.77</td>
<td>0.14</td>
</tr>
<tr>
<td>3</td>
<td>37.02</td>
<td>37.27</td>
<td>37.19</td>
<td>37.16</td>
<td>0.13</td>
</tr>
<tr>
<td>4</td>
<td>35.93</td>
<td>36.15</td>
<td>36.1</td>
<td>36.06</td>
<td>0.13</td>
</tr>
<tr>
<td>5</td>
<td>36.66</td>
<td>36.86</td>
<td>36.82</td>
<td>36.80</td>
<td>0.14</td>
</tr>
<tr>
<td>6</td>
<td>37.73</td>
<td>37.96</td>
<td>37.92</td>
<td>37.88</td>
<td>0.14</td>
</tr>
</tbody>
</table>

**Average = 0.14**

**Total Ash = average X 100/ Wt. of sample taken**

**Total Ash = 0.14X100/2 = 7.06%**

### Acid insoluble ash

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Initial weight</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36.07</td>
<td>36.06</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>36.80</td>
<td>36.77</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>37.88</td>
<td>37.85</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**Average = 0.07**

**Acid insoluble ash = average X 100/ Wt. of sample taken**

**Acid insoluble ash = 0.07 X100/2 = 3.5%**

### Water insoluble ash

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Initial weight</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39.07</td>
<td>38.98</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>37.77</td>
<td>37.68</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>37.16</td>
<td>37.07</td>
<td>0.09</td>
</tr>
</tbody>
</table>

**Average = 0.09**

**Water insoluble ash = average X 100/ Wt. of sample taken**

**Water insoluble ash = 0.09X100/2 = 4.5%**

### Extractive value

#### Ethyl alcohol soluble extractive value

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Weight of petridish</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.23</td>
<td>16.242</td>
<td>0.0038</td>
</tr>
<tr>
<td>2</td>
<td>16.69</td>
<td>16.70</td>
<td>0.0139</td>
</tr>
<tr>
<td>3</td>
<td>16.06</td>
<td>16.07</td>
<td>0.0137</td>
</tr>
</tbody>
</table>

**Average =0.010**

**Alcohol Soluble Extractive = average value X 500**

**Alcohol Soluble Extractive =0.010X500 =5.23%**

#### Water soluble extractive value

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Weight of Petridis</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.79</td>
<td>14.82</td>
<td>0.031</td>
</tr>
<tr>
<td>2</td>
<td>16.22</td>
<td>16.25</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>16.44</td>
<td>16.47</td>
<td>0.03013</td>
</tr>
</tbody>
</table>

**Average =0.022**

**Water Soluble Extractive = average value X 500**

**Water Soluble Extractive =0.022X500 =11.42%**

### Chloroform soluble extractive value

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Weight of Petridis</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14.7931</td>
<td>14.7969</td>
<td>0.0038</td>
</tr>
<tr>
<td>2</td>
<td>14.9201</td>
<td>14.9238</td>
<td>0.0037</td>
</tr>
<tr>
<td>3</td>
<td>14.3309</td>
<td>14.3331</td>
<td>0.0022</td>
</tr>
</tbody>
</table>
Average =0.003
Chloroform Soluble Extractive= average value X 500
= 0.003X500
= 1.61%

Methanol soluble extractive value

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Weight of Petridis</th>
<th>Final weight</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.46</td>
<td>32.49</td>
<td>0.032</td>
</tr>
<tr>
<td>2</td>
<td>32.53</td>
<td>32.61</td>
<td>0.0022</td>
</tr>
<tr>
<td>3</td>
<td>32.26</td>
<td>32.29</td>
<td>0.0282</td>
</tr>
</tbody>
</table>

Average = 0.018
Alcohol Soluble Extractive= average value X 500
= 0.018X500
= 9%

Phytochemical parameter

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Constituent</th>
<th>Reagent</th>
<th>Observation</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloid</td>
<td>Wagner reagent</td>
<td>Yellow</td>
<td>Present</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>Fehling solution</td>
<td>Brick red</td>
<td>Present</td>
</tr>
<tr>
<td>3</td>
<td>Flavanoid</td>
<td>Sample+ HCl</td>
<td>No colour</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Protein</td>
<td>Aq. Sample +NaOH+CuSO4</td>
<td>Green colour</td>
<td>Absent</td>
</tr>
<tr>
<td>5</td>
<td>Resin</td>
<td>Sample +acetone+ distilled water</td>
<td>Turbidity present</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Saponin</td>
<td>5ml sample+1 drop sodium bicarbonate</td>
<td>Comb like froth</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Steroid</td>
<td>Slowsky reaction</td>
<td>Red colour is formed in chloroform layer</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Tannin</td>
<td>2ml sample+ few drop ferric chloride</td>
<td>Green colour</td>
<td>Present</td>
</tr>
<tr>
<td>9</td>
<td>Starch</td>
<td>0.015gm+0.075gm KI +distilled water+aq sample</td>
<td>Green colour</td>
<td>Absent</td>
</tr>
</tbody>
</table>

High Performance Thin Layer Chromatography
The respective peak area was recorded and calibration curve was prepared by plotting peak area vs. concentration of reserpine applied (fig 4) calculated amount of reserpine present in the sample from the calibration curve. The percent of reserpine was 0.59.
254 nm (Before derivatization)
Table 12
254 nm (Before derivatization)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Rf value</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Track 1</td>
<td>0.82 (reserpine)</td>
<td>Blue</td>
</tr>
<tr>
<td>Track 3</td>
<td>0.27 (reserpine)</td>
<td>Grey</td>
</tr>
<tr>
<td>Track 4</td>
<td>0.40</td>
<td>Grey</td>
</tr>
<tr>
<td>Track 5</td>
<td>0.58</td>
<td>Grey</td>
</tr>
<tr>
<td>1</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.82</td>
<td></td>
</tr>
</tbody>
</table>

Table 13
Visible light (after derivatization)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Rf value</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.13</td>
<td>Yellowish brown</td>
</tr>
<tr>
<td>2</td>
<td>0.58</td>
<td>Yellowish brown</td>
</tr>
</tbody>
</table>

Fig.4: A calibration curve for reserpine by HPTLC

Fig.5: A spectra comparison of reserpine standard with reserpine in sample(sarpagandha)
DISCUSSION

The plant *Rauwolfia serpentina* belonging to family Apocynaceae is important folklore medicine as well as modern medicine system. The root of this species are mainly explored rather than other parts. The phytochemical screening of *Rauwolfia serpentina* root extract shows presence of various bioactive compound like alkaloid, carbohydrate, resin, saponin, steroid, tannin, while flavanoid, protein were absent in root of *Rauwolfia serpentina*. The physicochemical evolution like ash value, extractive value LOD are parameter of standardization of plant. Total ash of *Rauwolfia serpentina* root was 7.04%, water soluble extractive value was 11.41% and ethyl alcohol soluble extractive value was 5.23% and methyle alcohol soluble extractive value was 9% and chloroform soluble extractive value was 1.62%. Acid insoluble ash value was 4.5%. LOD value of *Rauwolfia serpentina* was found 7.5%. Powder microscopy of *Rauwolfia serpentina* root powder shows various structure which help to identify the plant property like lignified tissue, cork cell, xylem, phloem, starch grains, wood parenchyma. The analytical data as High Performance Thin Layer Chromatography shows Rf value 0.82 (reserpine) and the amount of reserpine was found 0.59%. The U.V. spectrophotometer revealed that 3.37% tannin is found in *Rauwolfia serpentina*.

CONCLUSION

Due to high demand over the world market the genuine plant (*Rauwolfia serpentina*) is almost on the track of extinction and in future can be categorized as endangered species. Therefore it is necessary to search the content of reserpine to other part of different species rather than root.

Acknowledgement

I would like to thank Dr. Vandna Pathak Associate Professor and I also expresses my sincere gratitude to Dr. Manoj Tripathi HOD of Ayurved Sadan Arogyadham DRI Chitrakoot Satna for guidance and support throughout the work.

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