ABSTRACT

Position of Rauwolfia in industry is emerging. Reserpine is the first herbal constituent included in modern medicine system. Due to its high demand over the world market the genuine plant (i.e R.serpentina Linn. benth.ex kurz) is almost on The track of extinction and in future can be categorized as an endangered species. Therefore The present study was attempted to search reserpine from other parts of R.serpentina and R. tetraphylla. So both the species can be explored for the isolation of bioactive reserpine and the commercial plant R. Serpentina can be minimized from over exploitations and extinction. And also establish the various Pharmacognostical parameters of Rauwolfia species of Eastern Odisha for their correct identifications.

Key words: UV Spectroscopy, Reserpine, R. serpentina, R. tetraphylla.

INTRODUCTION

The tribal areas of Baipariguda,Koraput (District), Orissa. due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. The various species of Rauwolfia (Apocynaceae) are widely distributed. Several Rauwolfia species in India are known to possess ethno medicinal and folklore claims Rauwolfia species are very important due to their traditional medicinal use such as insanity, edema, Rheumatic pain, Epilepsy, Snake and Scorpio bite, Purgative, Sedative, Anthelmintic, relief cough, anti diarrhea and some intestinal disease due to the presence of Reserpine [1] Reserpine is also important in modern medicine system to treat a number of diseases like hypertension, neuropsychiatry disorder and as tranquilizer[2,3] Reserpine was the first herbal constituent included in modern medicine system. Further, due to its high demand over the world market the genuine plant (i.e R.serpentina linn. benth. ex kurz) is almost on he track of extinction and in future can be catagorised as an endangered species. There fore it is necessary to search the contents of Reserpine from other parts of different species. Pharmacognostical investigation this explains of the plant not a broad but adequate for a medical practitioner, Pharmacognostical parameters of a species is need for their correct identifications[4]

MATERIALS AND METHODS

Collection of Plant Material
The fresh plant material of R.serpentina linn and R. tetraphylla. was collected from the tribal belts of the Baipariguda forest of Koraput districtin The month of august&September,The plant was identified, confirmed and authenticated by the taxonomist Dr.N.K.Dhal, Institute of Minerals and Materials Technology Bhubaneswar, Orissa
Preparation of Extract
The coarse powder was taken in Soxhlet apparatus and extracted successively with methanol. The extraction was done for 72 hours. The marc of each extract was dried and used for extraction with successive solvent. The liquid extracts were concentrated separately under vacuum and resulting extracts were kept in desiccator until further. [5,6]

Phytochemical investigation
Chemical tests were carried out on all the extracts (Methanolic extracts) for the qualitative determination of phytochemical constitute. [5,6]

Table 1: Preliminary phytochemical screening of leave, stem and roots of _R. Seprentina_ and _R. tetraphylla_.

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th><em>R. Seprentina</em></th>
<th><em>R. tetraphylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf</td>
<td>Stem</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Glycosides</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>reducing sugar</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>flavnoid</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Amino acids</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

[+ ++ Highly present, ++ Moderately present, + Slightly present, -ve Absent]

Table 2: Determination of Ash value of leave, stem and roots of _R. seprentina_ & _R. tetraphylla_.

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th><em>R. Seprentina</em></th>
<th><em>R. tetraphylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wt. of material (gms)</td>
<td>Wt. of as (gms)</td>
</tr>
<tr>
<td>Leaf</td>
<td>2</td>
<td>0.1578</td>
</tr>
<tr>
<td>Stem</td>
<td>2</td>
<td>0.1875</td>
</tr>
<tr>
<td>Root</td>
<td>2</td>
<td>0.1785</td>
</tr>
</tbody>
</table>

Physicochemical parameter
Determination of total ash
Total ash determination constitutes detecting the physiological ash (ash derived from plant(tissue) and nonphysiological ash (ash from extrageeous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material of each formulation and the individual ingredients of the powers were placed separately in a suitabledared crucible of silica previously ignited and weighed. The powdered drugs were spread into an even layer and weighed accurately. The materials were incinerated by gradually increasing the heat,
not exceeding 450°C until free from carbon, cooled in a desiccator, weighed and percentage ash was calculated by taking in account the difference of empty weight of crucible & that of crucible with total ash.[7,8]

**Determination of solvent Extractive value**

**Alcohol soluble extractive value**

5g of coarsely powdered air-dried drug was macerated with 100ml of alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly; taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish at 105°C to constant weight and weighed. The percentage of alcohol-soluble extractive was calculated with reference to the Mair-dried drug and is represented as % value.[7,8]

**Water soluble extractive value**

5g of coarsely powdered air-dried drug was macerated with 100ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. It was then filtered rapidly, taking precautions against loss of solvent. 25ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish at 105°C to constant weight and weighed. The percentage of water-soluble extractive was calculated with reference to the air-dried drug and is represented as % value.[7,8]

Table 3: Determination Extractive value of leave , stem and roots of *R. seprentina* & *R. tetraphylla*

<table>
<thead>
<tr>
<th>Powder material (w/w)</th>
<th><em>R. seprentina</em></th>
<th><em>R. tetraphylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Water soluble % ext. value (w/w)</td>
<td>18</td>
<td>26.2</td>
</tr>
<tr>
<td>Alcohol soluble % ext. value (w/w)</td>
<td>17.2</td>
<td>23.4</td>
</tr>
</tbody>
</table>

Table 4: Determination of loss of drying of leave , stem and roots of *R. seprentina* & *R. tetraphylla* Powder

<table>
<thead>
<tr>
<th>Powder material (gms)</th>
<th><em>R. seprentina</em></th>
<th><em>R. tetraphylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss in wt. % Loss in wt. (w/w)</td>
<td>0.1210 6.05</td>
<td>0.1245 6.22</td>
</tr>
</tbody>
</table>

**Loss on drying**

Loss on drying is the loss of mass expressed as percent w/w. About 10g of dug samples of each formulation was accurately weighed in a dried and tared flat weighing bottle and dried at 105C for 5hrs. Percentage was calculated with reference to initial weight.[7,8]

Table 4: Determination of loss of drying of leave , stem and roots of *R. seprentina* & *R. tetraphylla* Powder

<table>
<thead>
<tr>
<th>Powder material (gms)</th>
<th><em>R. seprentina</em></th>
<th><em>R. tetraphylla</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss in wt. % Loss in wt. (w/w)</td>
<td>0.1430 7.14</td>
<td>0.1350 6.75</td>
</tr>
</tbody>
</table>

**Powder microscopy of *R. seprentina* & *R. tetraphylla***

**Instrument used**: Leica microscope (EZ-4D)

**Magnification**: 40x × 2.5

**Procedure:**

Powder microscopy is an important parameter for identification of the drug. A judicious quantity of powder(leaf,stem,&root) was taken on a glass slide to which are added a few drops of chloral hydrate and was heated for 1-2 min, After placing a cover slip ,care should be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the coverslip . Excess of chloral hydrate outside the coverslip is to be withdrawn using a blotting paper(Chloral hydrate is used to clear the tissues and to bring in clarity of the view) Lignified tissue
are to be confirmed by staining. To the powder a few drops of mixture of 1:1 Phloroglucinol + Conc HCl was added and after 3 to 4 minutes observed under microscope. The well known identifying characters were determined under Leica microscope (10 x & 40x) [9,10]

**R. sepretina Stem Powder**

![Fig1a: Lignified fiber](image1)

![Fig1b: Ston cell](image2)

![Fig1c: Xylum vessels](image3)

**R. sepretina Leaf Powder**

![Fig1d: Trichome](image4)

![Fig1e: Anisocytic stomata](image5)

**R. sepretina Root Powder**

![Fig1f: Cork cell](image6)

![Fig1g: Stone cell](image7)
Histological study

Instrument used: Leica microscope (EZ-4D)
Magnification: 40x × 2.5

Procedure:
For Anatomical study invariably slides were prepared. A transverse section of required parts (leaf, bark, & root) of R. serpentina and R. tetraphylla was taken on a glass slide to which are added a few drops of chloral hydrate and is heated for 1-2 minute then two drop of phloroglucinol was added followed by one drop conc. Hcl. Then mounted with glycerine. Care how ever is to be taken to avoid air bubbles and to see that there is sufficient chloral hydrate under the cover slip. Excess of chloral hydrate out side the cover slip is to be withdrawn using a blotting paper. Chloral hydrate was used to clear the tissues and to bring in clarity of the view. The well known identifying characters were determined under Leica microscope (10 x & 40x).[11]
Fig3a: T.S of Leaf (R. serpentina)

Fig3b: T.S of stem (R. serpentina)

Fig3c: T.S of root (R. serpentina)
The analytical data such as TLC revealed that Reserpine is present in other parts (leaf, stem) rather than the root of both the species. [12]

**TLC Profiles Studies**

The analytical data such as TLC revealed that Reserpine is present in other parts (leaf, stem) rather than the root of both the species. [12]
Fig4a: Development of TLC finger print profile at 366nm

Solvent system:(Toluene:ethyl acetate:diethyl amine=7:2:1)

Fig4b: Development of TLC finger print profile at 254nm

Solvent system:(Toluene:ethyl acetate:diethyl amine=7:2:1)

Quantitative Determination of Reserpine in different parts of Rauwolfia serpentina and R. tetraphylla by UV Spectroscopy

Procedure:
1mg of reserpine was taken and dissolve in 10ml of methanol, and various dilution are made from it having concentration(2µg/ml-10 µg/ml).1mg of methanolic extract of R.serpentina and R. tetraphylla was taken and dissolve in 10ml of methanol, and various dilution are made from it having concentration (5 µg/ml). all the various dilutions of reserpine were observed under UV spectrophotometer using λmax 268nm. Absorbance of all the samples and standard was calculated.

Method:
In the proposed UV method the reference standard two milliliters of reserpine solution containing between 2-10 microgram of reserpine is pipette in to a test tube, like this the samples were prepped 5 micrograms per ml.absorbance taken at 268nm. The experiment was don in triplicate Calibration curves A series of standard curves were prepared over a concentration range 2-10µg(n=3,five standards) The data of concentration versus absorbance was btreated by liner testsquare regression analysis.[13,14]
Analysis of different sample
5 µgm of each sample were analysed by the proposed method, then by extrapolating from the absorbance data the unknown concentration were determind.

Validation of the method accuracy
Accuracy
The accuracy of an analytical procedure is the closeness of test results obtained by that procedure to the true value. The accuracy of an analytical procedure should be established across its range. Accuracy can be accomplished in a variety of ways, including evaluating the recovery of the analyte (present recovery) across the range of the assay, or evaluating the linearity of the relationship between estimated and actual concentration to the pre analysed sample. 1 mg of reserpine was added and the mixture was analysed by the proposed method. The experiment was conducted in triplicate to check recovery and accuracy of the system [14].

Precision validation
Precision was performed by taking 6 samples each from the stock solution of reserpine (100 µg) and analysed with the proposed method and also the interday and interaday precision were determined for various samples.

Linearity
According to ICH guidelines, the linearity of an analytical method is its ability (within a given range) to obtain the test result that are directly proportional to the concentration of the sample. Linearity test was performed using five different amounts of reserpine in the range of (2-10 µg/ml). Solution corresponding to each concentration level were analysed by the proposed method in triplicate [14].

Limit of detection and quantitation
These parameters were calculated from the data set obtained from a linear calibration curve in the range (1-10 µg) two replicates each standard for this purpose, a 1 µl volume of the corresponding standard solution was tested in duplicate and analysed with the proposed method. the corresponding slope and regression standard deviation (SY/X) values were used to establish sensitivity (SY/X/b). LOD & LOQ was determined.

Graph 1: Calibration plot of Reserpine

![Calibration plot of Reserpine](image_url)
Table 5: Absorbance of Reserpine at various concentration

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>0.034±0.00152</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>0.062±0.00152</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>0.104±0.00200</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0.136±0.01700</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>0.170±0.03520</td>
</tr>
</tbody>
</table>

Analysis of different samples

With respect to absorbance of different samples the unknown concentration were determined by extrapolation

Table 6: Absorbance and content of reserpine in *R. serpentina* and *R. tetraphylla*

<table>
<thead>
<tr>
<th>Methanolic Extract.</th>
<th>Concentration (µg/ml)</th>
<th>Absorbance</th>
<th>Conc. of Reserpine (µg/ml)</th>
<th>Absorbance</th>
<th>Conc. of Reserpine (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>5µg/ml</td>
<td>0.071</td>
<td>4.220</td>
<td>0.052</td>
<td>3.11</td>
</tr>
<tr>
<td>Stem</td>
<td>5µg/ml</td>
<td>0.045</td>
<td>2.596</td>
<td>0.040</td>
<td>2.40</td>
</tr>
<tr>
<td>Root</td>
<td>5µg/ml</td>
<td>0.009</td>
<td>0.700</td>
<td>0.007</td>
<td>0.47</td>
</tr>
</tbody>
</table>

Graph 2: Content of Reserpine in *R. serpentina* & *R. tetraphylla*

RESULTS AND DISCUSSION

The plant *R. serpentina* and *R. tetraphylla* belonging to Family Apocyanaceae are important Folklore medicine as well as in modern medicine system. The root of this species are mainly explored rather than other parts. The Phytochemical screening of Various extract of *R. serpentina* and *R. tetraphylla* shows presence of various bioactive compound such as alkaloid, glycoside, reducing sugar, steroid, & tannin were present in all parts of *R. serpentina* and *R. tetraphylla* flavonoid present in leaf & bark of both species except root. Amino acid, sapinin & triterpinoid were absent in both the species. Physicochemical evaluation like ash value, extractive value, moisture content are the parameter
of standardization of both the plants. Total ash value of the *R. serpentina* leaf, stem & root is found to be 7.88, 9.36, 8.92 & in *R. tetraphylla* found to be 6.67, 8.47, 7.90, watersoluble Extractive value of leave, stem and roots of both the species is found to be 18, 16, 5, 17.5 & 17.2, 16, 3, 18, alcohol soluble Extractive value of leave, stem and roots of both the species is found to be 26, 2.24, 5, 25.5 & 23.5, 22, 5, 21.5. Loss of drying of *R. serpentina* leaf, stem & root is found to be 6.05, 7.14, 8.34 & in *R. tetraphylla* leaf, stem & root is is found to be 6.22, 6.75, 7.64. Powder microscopy of both the species shows various structure which will help to identify the plants properly and help to check intermix of adulterants and differentiate it from the allied species. Lignified fiber, reddish color stone cell, xylem vessel were present in *R. serpentina* stem powder, multicellular trichome, & anisocytic stomata were present in leaf powder of *R. serpentina*. Non-stratified cork cells & horse shoe type of stone cell were present in *R. serpentina* root powder. In *R. tetraphylla* stem powder stone cell, xylem vessel & fiber vessel were present. Multicellular trichome, & anisocytic stomata were present in leaf powder of *R. tetraphylla*. Triangular stone cell & simple fiber were present in root powder of *R. tetraphylla*. The histological studies of *R. serpentina* root shows cork is made of thick stratified cells, pink color pheloderm is presents secondary xylem is characerised by straight rays. Stem part shows cortex consist of parenchymatous cell. Central part of stem occupied by pith, medullary rays are present, leaf shows trichome were present in both the epidermis, stomata are also present in both the epidermis, vascular bonds were cojoit & collateral. The histological studies of *R. tetraphylla* shows in root cork is made of rectangular cell, cortex cells were polygonal, the secondary xylem has numerous large vessels, stem shows cortex consist of parenchymatous cell, centra part occupied by pith medullary rays were present protoxylem & metaxylem also present, in leaf trichomes were numerous, stomata were present in both the epidermis, vascular bonds were cojoit & collateral arranged in a ring. The analytical data such as TLC shows Rf value 0.45 (standard) is found in both the species of plant *R. serpentina* and *R. tetraphylla* in all parts (leaf, stem & root). The UV revealed that Reserpine is present from other parts rather than the root of both the species. Coecntration of reserpine present in different parts of *R. serpentina* is 4.220(µgm/ml), 2.596(µgm/ml), 0.700(µgm/ml) in root, stem & leaf, same concentration of reserpine present in different parts of *R. tetraphylla* is 3.11(µgm/ml), 2.40(µgm/ml), 0.47(µgm/ml) in root, stem & leaf.

**CONCLUSION**

Due to its high demand over the world market the genuine plant (*R. serpentina* linn. benth. ex kurz) is almost on the track of extinction and in future can be catagorised as an endangered species. Therefore it is necessary to search the contents of reserpine from other parts of different species rather than root. The proposed UV spectroscopy method used for the quantitative determination of reserpine in different parts of *R. serpentina* and *R. tetraphylla*. The roots of this species are mainly explored rather than other parts. The analytical UV spectroscopy data revealed that Reserpine is present in leaf, stem and root of both the species. So other parts of both the species can be explored for the isolation of bioactive compound reserpine. Further the commercial plant *R. Serpentina* can be minimized from over exploitations and extinction.

**Acknowledgement**

Authors wish to thank to local people of Baipariguda of Koraput and the taxonomist Dr. N.K. Dhal, Institute of Minerals and Materials Technology Bhubaneswar, Orissa, India for providing valuable information about the plant and its identification. The author wish to express their gratitude to Jeypore College of Pharmacy, Rondapalli, Jeypore, Koraput, Odisha, & The authors are thankful to the Dean, BIT Mesra, Ranchi for providing instrumental facility and other necessary support to carry out the experiment.

**REFERENCES**