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Phyto-pharmacognostical studies and quantitative determination of reserpine in different parts of *Rauwolfia* (spp.) of Eastern Odisha by UV Spectroscopy Method

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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.exkurz) 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 Estern 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, Anthemilatic,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. exkurz) is almost on he track of extinction and in future can be catagorised as an endangered species. There fore it is necessary to search thecontents 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 augest&September,The plant was identified, confirmed and authenticated by the taxonomist Dr.N.K.Dhal, Institute of Minerals and Materials Technology Bhubaneswar, Orissa

,India ,Vide Voucher Specimen no. (V.N. no-16,507)was submitted. After authentification leaf,stem,&root were collected in bulk and washed under running tap water to remove adhering dirt. Then shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder.

Instrument and Chemicals

U.V spectrophotometer (Systronics 105)
Leica microscope (EZ-4D)
Reserpine (standard)was procured from Sigma Aldrich, Banglore. Other chemicals used in the experiment (methanol) were of analytical grade and procured from Nice ChemicalLtd. Cochin, India.

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.*

	R. seprentina.			*R. tetraphylla*		
	Leaf	Stem	Root	Leaf	Stem	root
Alkaloid	+++	+++	+++	+++	+++	+++
Glycosides	+ +	+	-ve	+ +	+	-ve
Steroids	+	+	+++	+	+	++
reducing sugar	+	+	++	+	+	+
flavnoid	++	+	-ve	++	+	-ve
Amino acids	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	-ve	-ve	-ve	-ve	-ve	-ve
Tannins	+	+	+	+	+	+
Triterpenoid	-ve	-ve	-ve	-ve	-ve	-ve

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

Table 2: Deteramination of Ash value of leave , stem and roots of *R. seprentina*& *R. tetraphylla*

	R. seprentina.			*R. tetraphylla*		
	Wt. of material (gms)	Wt. of ash (gms)	% Total ash (w/w)	Wt. of material (gms)	Wt. of ash (gms)	% Total ash (w/w)
Leaf	2	0.1578	7.88	2	0.1324	6.67
Stem	2	0.1875	9.36	2	0.1752	8.47
Root	2	0.1785	8.92	2	0.1643	7.90

Physicochemical parameter

Determination of total ash

Total ash determination constitutes detecting the physiological ash (ash derived from plant(tissue) and nonphysiological ash (ash from extrageneous matter, especially sand and soil adhering to the surface of the drug). For its detection, 2g of powdered material of eachformulation and the individual ingredients of the powers were placed separately in a suitabletared crucible of silica previously ignited and weighed. The powdered drugs were spread into aneven layer and weighed accurately. The materials were incinerated by gradually increasing theheat,

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 airdried 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 tothe air-dried drug and is represented as % value.[7,8]

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

	***R. seprentina*.**		***R. tetraphylla***	
Powder material	**Water soluble % ext.value (w/w)**	**Alcohol soluble % ext.valu (w/w)**	**Water soluble % ext.value (w/w)**	**Alcohol soluble % ext.value (w/w)**
Leaf	**18**	**26.2**	**17.2**	**23.4**
Stem	**16.5**	**24.5**	**16.3**	**22.5**
Root	**17.5**	**25.5**	**18**	**21.2**

Loss on drying

Loss on dying 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: Deteramination of loss of drying of leave , stem and roots of *R. seprentina*& *R. tetraphylla Powder*

	***R. seprentina*.**		***R. tetraphylla***	
powder material	**Loss in wt. (gms)**	**% Loss in wt. (w/w)**	**Loss in wt. (gms)**	**% Loss in wt. (w/w)**
Leaf	**0.1210**	**6.05**	**0.1245**	**6.22**
Stem	**0.1430**	**7.14**	**0.1350**	**6.75**
Root	**0.1670**	**8.34**	**0.1530**	**7.64**

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.seprentina Stem Powder

Fig1a: Lignifiedfiber

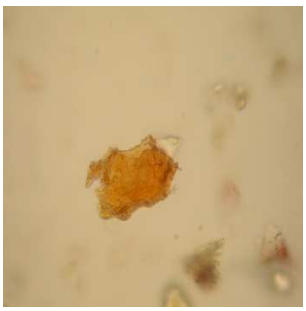


Fig1b:Ston cell

Fig1c:Xylum vessels

R.seprentina Leaf Powder

Fig1d: Trichome

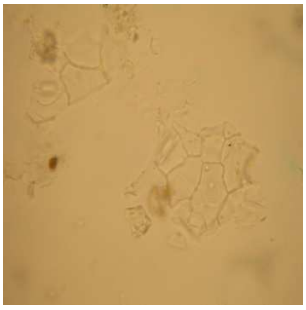


Fig1e: Anisocytic stomata

R.Seprentina Root Powder

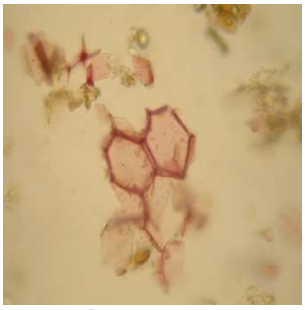


Fig1f: Cork cell

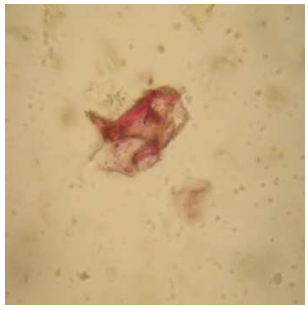


Fig1g: Stone cell

R. tetraphylla Stem Powder

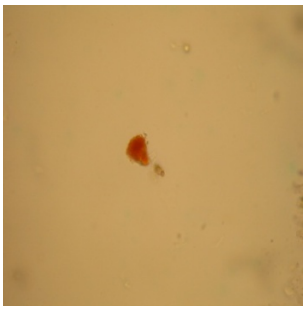


Fig2a: Stone cell

Fig2b: Fibre vessels

Fig2c: Xylem vessels

R. Tetraphylla Leaf Powder

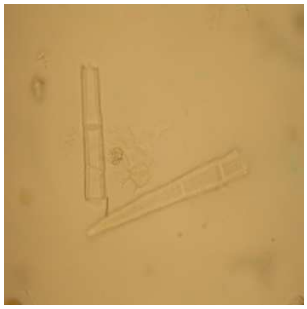


Fig2d: Trichomes

Fig2e: Anisocytic stomata

R. Tetraphylla Root Powder

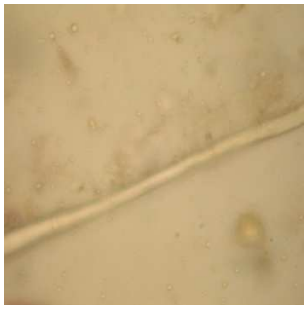


Fig2f : Simple fibre

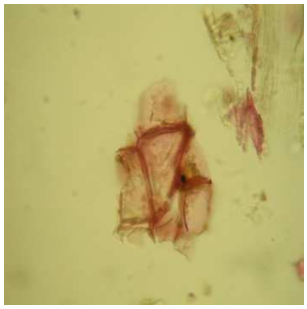


Fig2g: Triangular stone cell

Histological study
Instrument used : Leica microscope (EZ-4D)
Magnification :40x × 2.5
Procedure:
For Anatomical study invariably slides were prepared. A tansverse 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*)

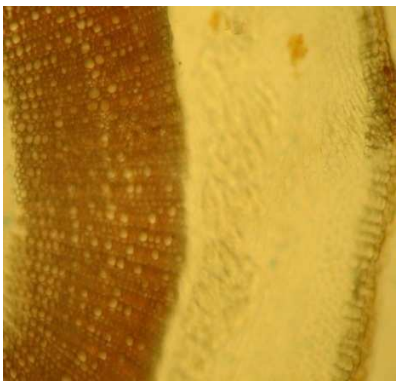


Fig3b:T.S of stem (*R.serpentina*)

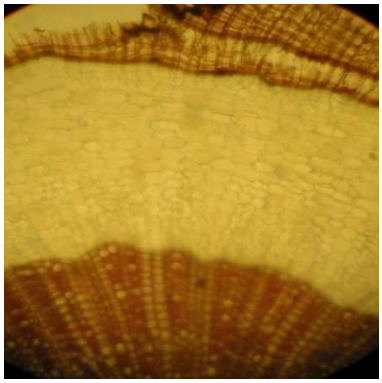


Fig3c:T.S of root (*R.serpentina*)

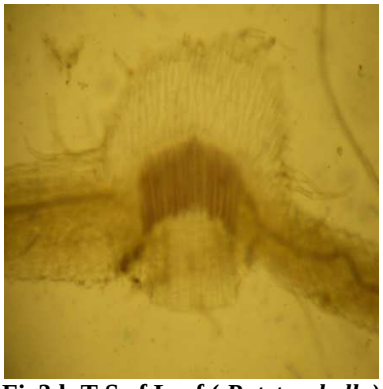


Fig3d: T.S of Leaf (*R. tetraphylla)*

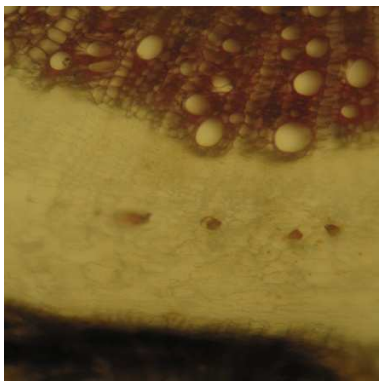


Fig3e: T.S of Stem (*R. tetraphylla)*

Fig3f: T.S of Root (*R. tetraphylla)*

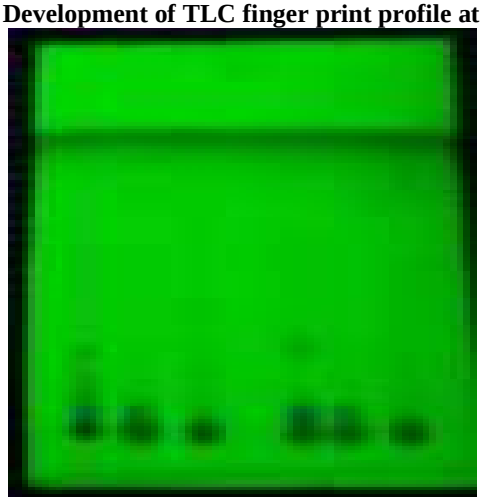
TLC Profiles Studies

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(2µmg/ml-10 µmg/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 µmg/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 methodthe 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

5µgm of each sample were analysed by the proposed method, then by extraploting from the absorbance data the unknown concentration were determind.

Validation of the method accuracy

Accuracy

The accuracy of an analytical procedure 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 procedureshould be established across it range. Accuracycan be accomplished in avariety 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 analysedsample,1mg of reserpine was added and the mixture wasanalysed 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(100µg) and analysed with the proposed method and also the interday and interaday precision were determind for various samples

Linearity

According to ICH guidelines ,the linearity of an analyticalmethod is its ability(with in a given range) toobtain the test result that are directly proportional to the concentration of thesample. Linearity test was perfomed using five different amounts of reserpine in the range of (2-10 µmg/ml).solution corresponding to each concentration level were analysed by the proposed method in triplicate.[14]

Limit of detection and quantitation

These parameter s were calculated from the data set obtained from a linear calibration curve in the range (1-10µg) two replicates each standard for this purpose, a 1µl 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.

Graph1: Calibration plot of Reserpin

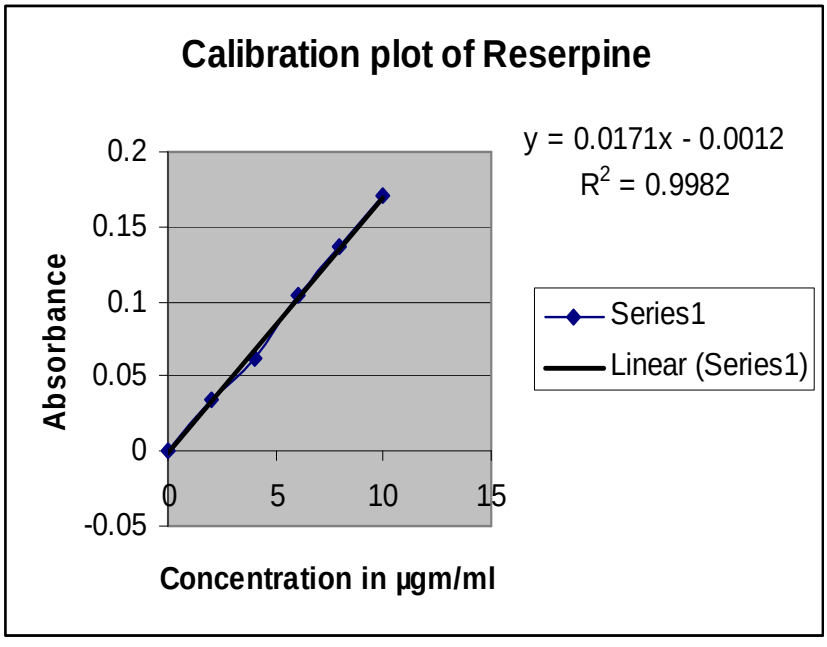


Table 5 :Absorbance of Reserpine at various concentration

Serial No.	Concentration(µg/ml)	Absorbance±SD
1	**2**	**0.034±0.00152**
2	**4**	**0.062±0.00152**
3	**6**	**0.104±0.00200**
4	**8**	**0.136±0.01700**
5	**10**	**0.170±0.03520**

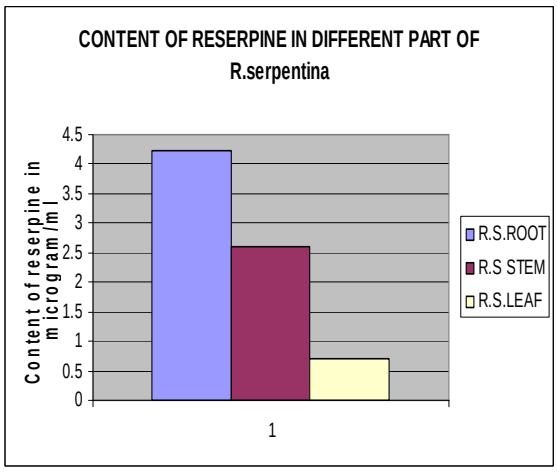
Analysis of different samples

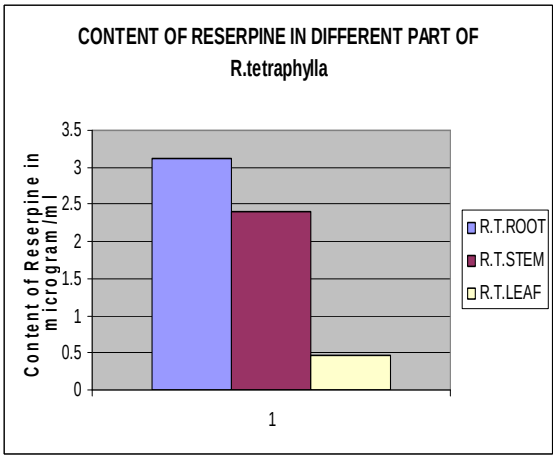
With respect to absorbance of different samples the unknown concentration were determind by extraplotion

Table6: Absorbance and content of reserpine in *R.serpentina* and *R.tetraphylla*

		R.serpentina		***R.tetraphylla***	
Methanolic Extract.	**Concentration**	**Absorbance**	**Conc. of Reserpine (µgm/ml)**	**Absorbance**	**Conc. of Reserpin (µgm/ml)**
Root	5µgm/ml	0.071	4.220	0.052	3.11
Stem	5µgm/ml	0.045	2.596	0.040	2.40
Root	5µgm/ml	0.009	0.700	0.007	0.47

Graph 2: Content of Reserpine in *R. seprentina*& 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 variou bio active 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 ,sapnin&triterpinoid were abscent 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.alcoholsoluble Extractive value of leave, stem and roots of both the species is found to be26.2,24.5,25.5&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 cheak intermix of adulterants and differentiate it from the allied species. Lignified fiber,reddish colour 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 cell&horse shoe type of stone cell were present in *R.serpentina* root powder. in *R.tetraphylla* stem powder stone cll,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 colour pheloderm is presentsecondary xylem is charerised 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* showesin root cork is made of rectangular cell,cortex cellwere hexagonal the secondary xylem has numerous large vesselsstem shows cortex cosist of parenchymatous cell,centra part occupied by pith medullary rays were present protoxylem&metaxylem also present..in leaf trichomes were numerious,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. Cocentration 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 Cocentration of reserpinepresent 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(i.e R.serpentina linn. benth. exkurz) is almost on the 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 rather than root.The proposed UVspetroscopy 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 UVspetroscopy 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.

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