Original Article   **Open Access**

Estimation of reserpine from healthy and diseased roots of *Rauwopfia serpentina* (L.) Benth. Ex. Kurz (Sarpagantdha) from different isolates by HPTLC analytical method

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ABSTRACT

Rauwolfia serpentina is an important medicinal herb used in Ayurveda and Alleopathy. Reserpine is an indole alkaloid present in *Rauwolfia serpentina* viz. reported to possess anti-hypertensive and tranquilizer property. Evaluation of herbal drug based on the amount of active constituent. Reserpine is present in all plant parts, but more in roots. Various factors are responsible for growth of plants and active constituent present in it. Roots are infected by fungi causing root rot disease that affect active constituent of root. Among the fungi, *Macrophomina phaseolina* causes severe root rot disease .In order to Changes in reserpine from healthy and infected roots of *Rauwopfia serpentina,* the healthy and infected roots of Sarpagandha was collected from four different places namely M. A., University, Parbhani designated HRS-1, IRS-1, Medicinal plant garden, M.P.K.V., Rahuri, designated HRS-2, IRS-2, Nagarjun medicinal plant garden P. D. K. V., Akola designated HRS-3, IRS-3 and S. G. M., Amravati University, Amravati designated HRS-4, IRS-4 during rainy season in the month of August 2009 was used for the analysis. In the present study, estimation of reserpine from healthy and infected roots of *Rauwopfia serpentina* was carried out by HPTLC analytical method. It was observed that there is decrease in reserpine content in infected roots.

Keywords: *Rauwolfia serpentina*, Root, Reserpine, HPTLC, Macrophomina *phaseolina*.

INTRODUCTION

The *Rauwolfia serpentina* Benth ex Kurze (family: Apocynaceae) is important medicinal herb used in Ayurveda, Siddha, Unani and Western system of medicines (Quareshi and Nawaz, 2009). Various alkaloids are present in different parts of plant viz. root, stem and leaf. Several alkaloids have been,

isolated from root bark of this plant including reserpine, Ajmaline, ajmalicine, yohimbine, etc. This plant is extensively used in the treatment of insanity and snake bite (Kokate and Purohit, 2003). The root extract is very useful in disorders of gastro intestinal tract viz., diarrhea, dysentery, cholera and colic (Quareshi and Nawaz, 2009). Reserpine is an Indole alkaloid used in lowering blood pressure[7-8], as tranquilizer[7-8] etc. Many methods like UV spectroscopy[2], HPLC[2], HPTLC[2], gas chromatography[5], voltametry[5], polarography[5], room temperature phosphometry[5] and spectrofluorimetry[5], are used for the determination of Reserpine in pharmaceutical preparations either in bulk, dosage forms or in biological fluids. Many of these methods cannot be used for the determination of reserpine in extracts due to the interference of other constituents of plant. The present study reporting HPLC method for detection of reserpine from Rauwolfia *serpentina* with validation data.

MATERIAL AND METHODS

Collection of Plant material and estimation of reserpine:
The estimation of reserpine of four healthy and four infected root samples of Sarpagandha i.e. collected during rainy season in the month of August 2009 from different places was performed at Ancrome test Lab. Pvt. Ltd., Mumbai.

The estimation of reserpine content in different root samples was carried out by HPTLC method. The healthy and infected roots of Sarpagandha was collected from four different places namely M. A., University, Parbhani HRS-1, IRS-1, Medicinal plant garden, M.P.K.V., Rahuri, HRS-2, IRS-2, Nagarjun medicinal plant garden P. D. K. V., Akola HRS-3, IRS-3 and S. G. M., Amravati University, Amravati HRS-4, IRS-4 during rainy season in the month of August 2009 was used for the analysis.

Chromatographic condition:
The four samples were spotted in the form of band length 8.0 mm with the help of 100 µl sized syringe on silica gel 60 F 354 plates. Thickness of plate has 20cm x 10cm (E. Merck kGaA) using a Camag Linomat 5 sample applicator instrument. Before chromatogram-phy, plate was preliminarily washed with methanol

and activated at 110[0]c temperature for 5 minute in an oven.

Experimental conditions:

Analysis Report	
SOP document	
Validated Description:	Design
Analysis	L:\LAB-DATA\Lab 2013\combined data\RESERPINE 1
Created/used by	Anchrom Test Lab P. Ltd
Current user	Anchrom Test Lab P. Ltd

Stationary phase	
Executed by:	Anchrom Test Lab. P.Ltd.
Plate size (X x Y)	20.0 x 10.0 cm
Material	HPTLC plates silica gel 60 F 254
Manufacturer	E. MERCK KGaA
Batch	
GLP code	
Pre-washing	No
Modification	No

Definitions – Screening	
Executed by	Anchrom Test Lab P. Ltd.

Samples:
Infected 1
Healthy 1
Infected 2
Healthy 2
Infected 3
Healthy 3
Infected 4
Healthy 4
Std. reserpine

Substance name	Rf	Window size	Manufacturer	Batch Number	Expiry date	Product Number
Reserpine	0.46	0.600				

Sample application -	CAMAG Linomat 5
Instrument	CAMAG Linomat 5
"Manually set to Executed'" S/N * ( )	
Executed by	Anchrom Test Lab Pvt. Ltd.

Linomat 5 application parameters	
Spray gas:	Inert gas
Sample solvent type:	Methanol
Dosage speed:	150nl/s
Predosage volume:	0.2ul

Sequence	
Syringe size:	100 µl
Number of tracks:	11
Application position Y:	8.0 mm
Band length:	8.0 mm

No.	Appl. position	Appl. Volume	Vial #	Sample ID	Active
1	20.0 mm	10.0 µl	1	Infected 1	Yes
2	36.0 mm	10.0 µl	2	healthy 1	Yes
3	52.0 mm	10.0 µl	3	infected 2	Yes
4	68.0 mm	10.0 µl	4	healthy 2	Yes
5	84.0 mm	2.0 µl	5	std reserpine	Yes
6	100.0 mm	5.0 µl	5	std reserpine	Yes
7	116.0 mm	10.0 µl	5	std reserpine	Yes
8	132.0 mm	10.0 µl	6	infected 3	Yes
9	148.0 mm	10.0 µl	7	healthy 3	Yes
10	164.0 mm	10.0 µl	8	infected 4	Yes
11	180.0 mm	10.0 µl	9	healthy 4	Yes

Constant samples applications were done with application speed 150nl/second and space between two bands 10 mm. The length of each chromatogram band was 8 mm. The slit dimensions was kept 6 x 0.45 mµ and scanned with speed 20 mm/sec. The monochromatic band width was set at 20mµ. The tracks were scanned with the help of CAMAG TLC scanner. The mobile phase Toulin: Ethyl acetate: Dethylamine (7:2:1) was used. Linear ascending development was carried out in a 20cm x 10cm twin through glass chamber saturated with mobile phase. The time required in mobile phase was 30 min at room temperature (25 ±2 ^{0}c) for saturation at relative humidity 60 ^{0}c± 5. After development, TLC plate was dried in air or with the help of hair dryer. The scanning was performed with the help of Camag scanner "scanner-170422". Measurement mode was absorption at 254 nm wavelength controlled by Win Cat CAMAG software versions 1.3.4. The radiation source was use deuterium (D2) lamp emitting continuous UV spectrum between 190 and 400nm. The concentration of compound was determined from the intensity of the different light. Evaluation of chemical compound was carried by peak area with linear regression. Percentage of reserpine was calculated from peak area of reserpine with the help of formula.

10 µl volume of each sample was applied at position 20, 36, 52, 68, 84, 100, 116, 132, 148 and 180 mms along with standard reserpine on HPTLC pate by using camag applicator.

The chromatographic plates were developed by mobile phase and scanned with CAMAG TLC Scanner "Scanner_170422" S/N. Win cat software gives chromatogram of samples and standard 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11. In the form of track 1 to 11. The Chromatogram shows tracks of reserpine present in samples and standard reserpine track as shown in fig. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 & 11. Peak area of reserpine was also shown in table 1, 2, 3, 4, 5, 6, 7, 8, 9,10 and 11. The reserpine shows variation in peak area indicates variation in content in different samples. The first two chromatograms of each sample were indicating infected and healthy roots respectively.

Detection - CAMAG TLC Scanner Information	
Application position	8.0 mm
Solvent front position	80.0 mm
Instrument CAMAG TLC Scanner "Scanner_170422" S/N 170422 (2.01.02)	
Executed by	Anchrom Test Lab P. Ltd.
Number of tracks	11
Position of first track X	21.5 mm
Distance between tracks	16.0 mm
Scan start pos. Y	5.0 mm
Scan end pos. Y	85.0 mm
Slit dimensions	6.00 x 0.45 mm, Micro
Optimize optical system	Light
Scanning speed:	20 mm/s
Data resolution:	100µm/step

Measurement Table

Wavelength	254
Lamp	D2
Measurement Type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	373 V

Detector properties	
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	0
Analog Offset	10%
Sensitivity	Automatic (42)

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	10.8 mm
Track end position	60.7 mm
Display scaling	
	Automatic
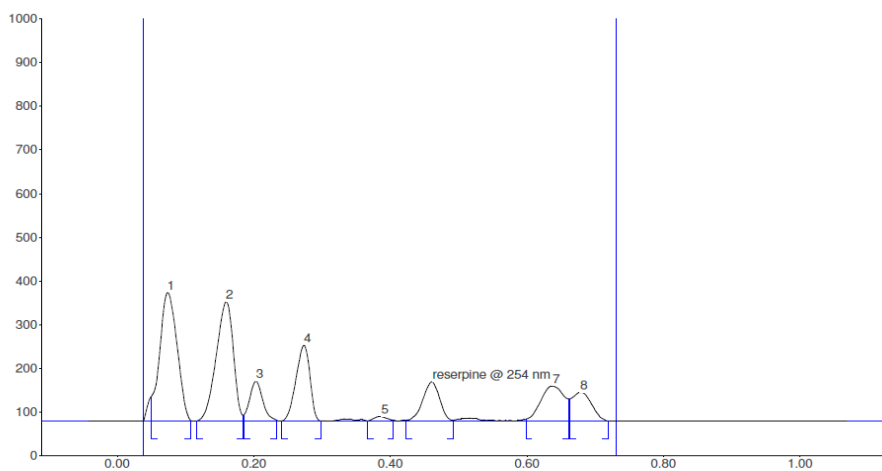
Fig. 1: Chromatogram of infected root sample

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	56.4	0.07	294.1	27.25	0.11	1.2	6306.9	27.99	unknown *
2	0.12	0.3	0.16	273.1	25.30	0.18	13.6	5756.4	25.55	unknown *
3	0.19	14.7	0.20	91.8	8.50	0.23	3.2	1473.0	6.54	unknown *
4	0.24	1.0	0.27	173.7	16.10	0.30	0.5	2974.8	13.20	unknown *
5	0.37	0.7	0.39	10.7	0.99	0.41	2.9	181.0	0.80	unknown *
6	0.42	2.7	0.46	89.9	8.33	0.49	2.3	1874.5	8.32	reserpine
7	0.60	5.1	0.64	80.6	7.47	0.66	51.2	2336.5	10.37	unknown *
8	0.66	51.5	0.68	65.5	6.07	0.72	0.9	1626.2	7.22	unknown *

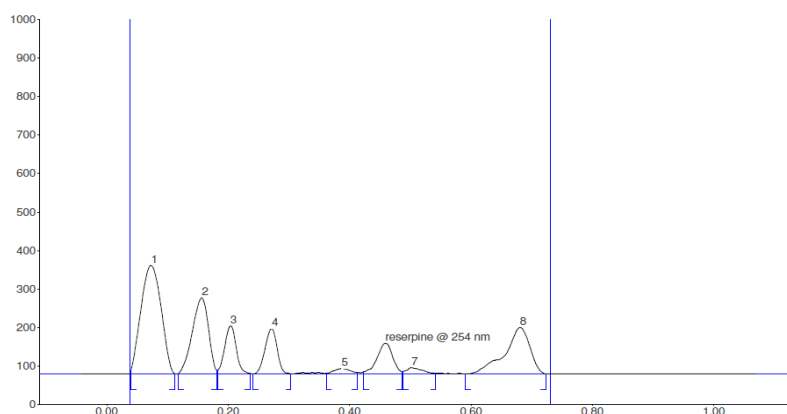**Table-1: Showing Peak area of reserpine infected root sample 1.**

Track 2, ID: healthy 1

Fig2: Chromatogram of Healthy 1 root sample

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	8.4	0.07	281.9	29.51	0.11	0.5	7824.8	34.27	unknown *
2	0.12	1.0	0.16	197.7	20.70	0.18	9.6	4401.1	19.28	unknown *
3	0.18	9.8	0.20	125.6	13.15	0.24	1.7	2000.0	8.76	unknown *
4	0.24	0.1	0.27	117.9	12.34	0.30	0.8	1962.6	8.60	unknown *
5	0.36	1.9	0.39	14.4	1.51	0.41	4.6	332.5	1.46	unknown *
6	0.42	5.8	0.46	80.3	8.41	0.49	6.5	1738.9	7.62	reserpine
7	0.49	6.7	0.50	16.6	1.74	0.54	1.6	372.3	1.63	unknown *
8	0.59	0.1	0.68	120.8	12.64	0.73	0.7	4200.0	18.40	unknown *

Table- 2: Showing Peak area of reserpine of healthy root sample 1
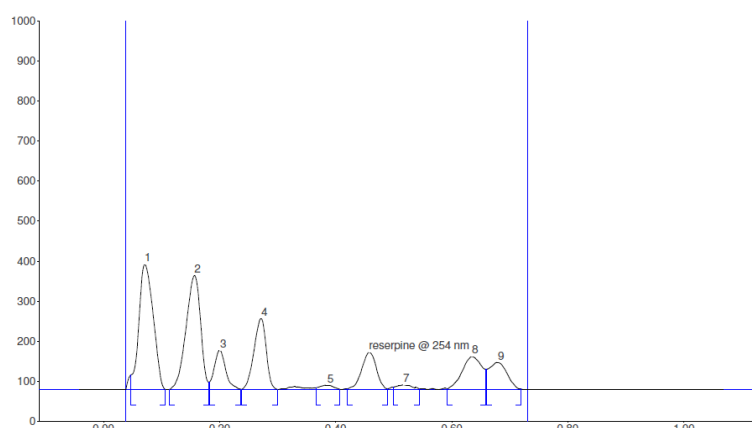
Track 3, ID: infected 2

Fig3: Chromatogram of infected root sample 2.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	36.2	0.07	312.6	27.45	0.11	0.4	6445.1	27.13	unknown *
2	0.11	0.1	0.16	284.9	25.01	0.18	18.5	5977.6	25.16	unknown *
3	0.18	19.3	0.20	97.8	8.59	0.24	0.9	1613.6	6.79	unknown *
4	0.24	1.0	0.27	177.4	15.58	0.30	0.4	3118.9	13.13	unknown *
5	0.37	4.8	0.39	11.2	0.98	0.41	0.9	222.3	0.94	unknown *
6	0.42	2.2	0.46	93.0	8.16	0.49	2.3	1975.4	8.32	reserpine
7	0.50	6.4	0.52	12.0	1.05	0.55	1.6	269.4	1.13	unknown *
8	0.59	2.2	0.64	82.4	7.24	0.66	51.2	2342.1	9.86	unknown *
9	0.66	51.2	0.68	67.7	5.94	0.72	1.8	1789.7	7.53	unknown *

Table -3: Showing Peak area of reserpine of infected root sample 2
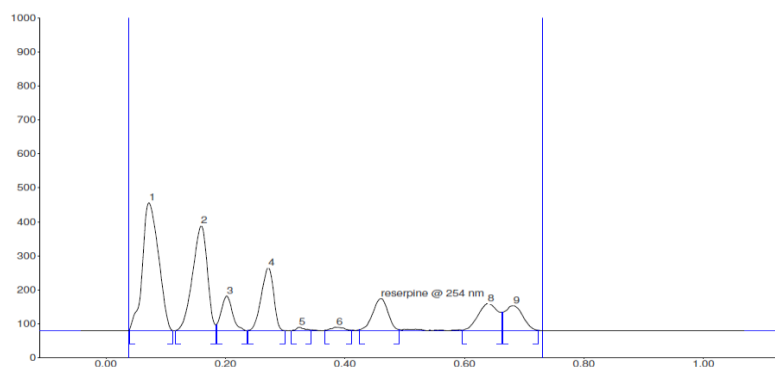
Track 4, ID: healthy 2

Fig. 4: Chromatogram of healthy root sample 2.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	4.9	0.07	376.7	30.35	0.11	0.1	8484.7	32.43	unknown *
2	0.12	0.2	0.16	308.1	24.82	0.18	17.3	6379.0	24.38	unknown *
3	0.19	19.3	0.20	102.1	8.22	0.24	0.8	1599.2	6.11	unknown *
4	0.24	1.5	0.27	185.0	14.90	0.30	0.5	3199.0	12.23	unknown *
5	0.31	1.0	0.32	10.5	0.84	0.34	2.5	126.7	0.48	unknown *
6	0.37	0.8	0.39	11.3	0.91	0.41	1.9	202.7	0.77	unknown *
7	0.43	3.3	0.46	94.7	7.63	0.49	4.4	2037.2	7.79	reserpine
8	0.60	2.0	0.64	79.2	6.38	0.66	54.6	2256.8	8.62	unknown *
9	0.67	54.7	0.68	73.7	5.94	0.73	2.0	1881.3	7.19	unknown *

Table-4: Showing Peak area of reserpine of healthy root sample 2
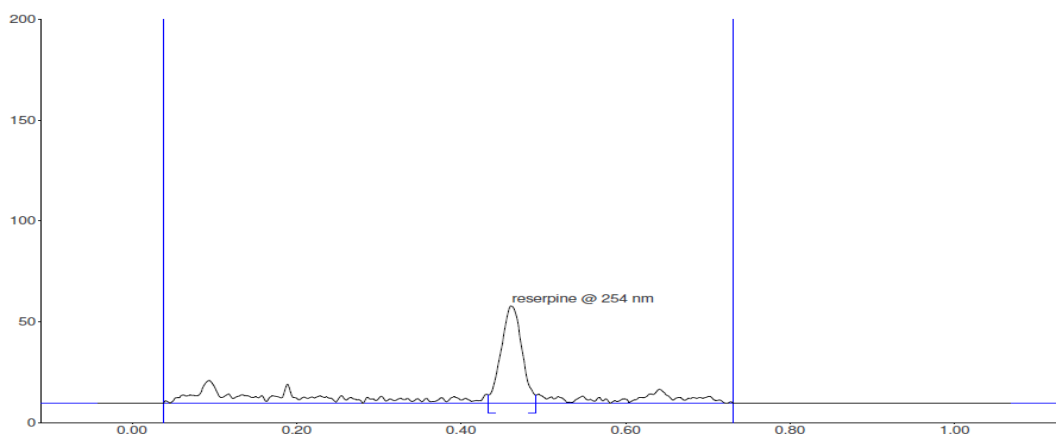
Track 5, ID: std reserpine

Fig.5: Chromatogram of standard reserpine.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.43	3.9	0.46	48.2	100.00	0.49	3.9	1005.7	100.00	reserpine

Table- 5: Showing Peak area of standard reserpine.
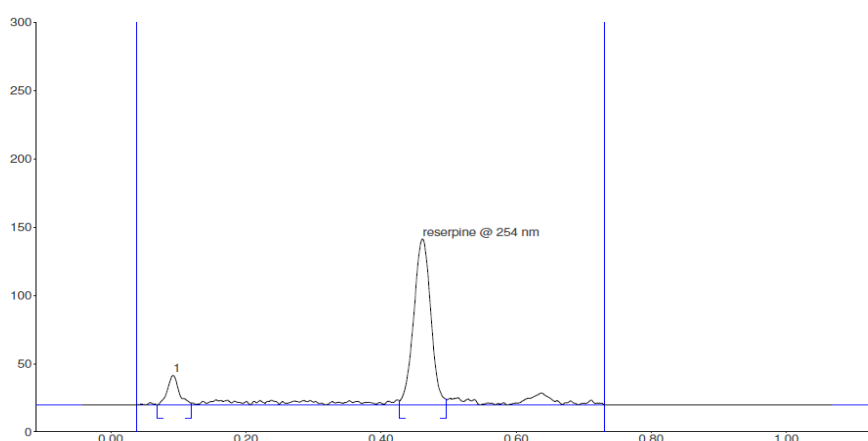
Track 6, ID: std reserpine

Fig.6: Chromatogram of standard reserpine.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	0.0	0.09	21.6	15.11	0.12	1.3	307.1	10.73	unknown *
2	0.43	3.2	0.46	121.6	84.89	0.50	4.1	2556.1	89.27	reserpine

Table -6: Showing Peak area of standard reserpine.
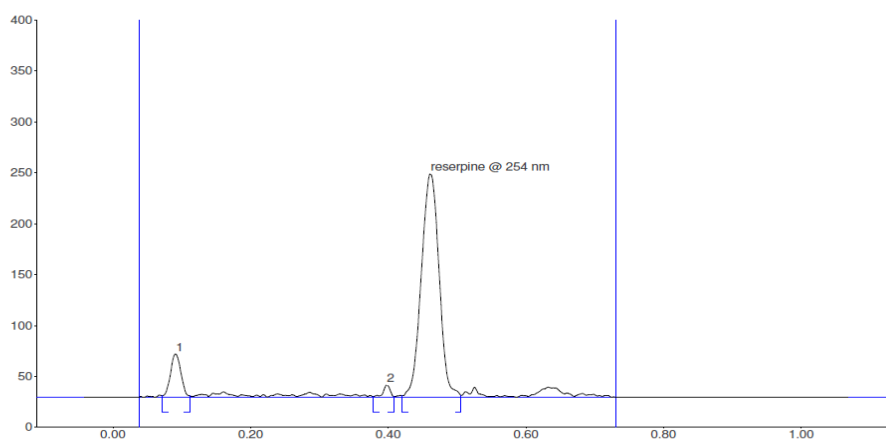
Track 7, ID: std reserpine

Fig. 7: Chromatogram of standard reserpine

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	1.2	0.09	42.3	15.46	0.11	1.1	536.6	10.01	unknown *
2	0.38	0.6	0.40	12.0	4.40	0.41	0.6	99.3	1.85	unknown *
3	0.42	1.4	0.46	219.4	80.14	0.51	1.6	4723.2	88.13	reserpine

Table- 7: Showing Peak area of standard reserpine.
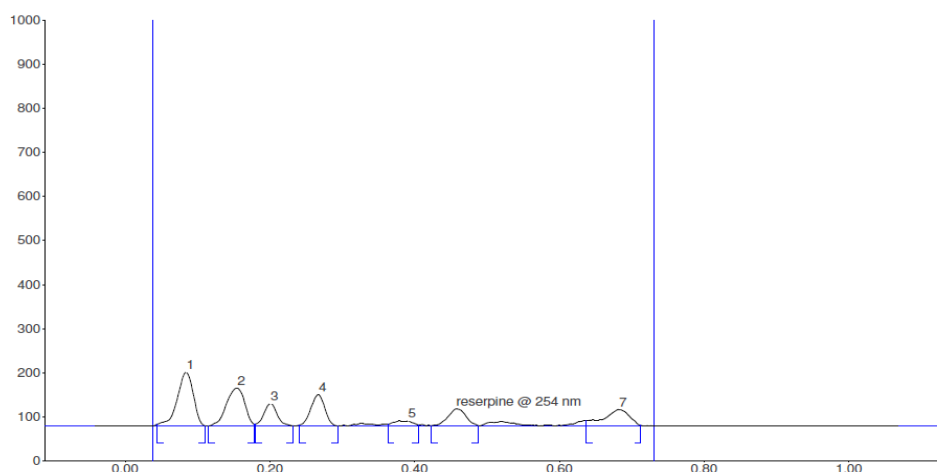
Track 8, ID: infected 3

Fig. 8: Chromatogram of infected root sample 3.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	3.7	0.08	121.7	29.19	0.11	0.1	2258.5	27.71	unknown *
2	0.12	0.4	0.16	85.8	20.57	0.18	4.1	1815.8	22.28	unknown *
3	0.18	4.2	0.20	50.8	12.19	0.23	0.7	774.1	9.50	unknown *
4	0.24	2.3	0.27	71.2	17.06	0.29	0.0	1143.4	14.03	unknown *
5	0.36	3.7	0.39	11.4	2.73	0.41	2.5	259.8	3.19	unknown *
6	0.42	0.5	0.46	38.8	9.31	0.49	0.7	803.8	9.86	reserpine
7	0.64	12.1	0.68	37.3	8.95	0.71	1.5	1096.0	13.45	unknown *

Table- 8: Showing Peak area of infected root sample 3.
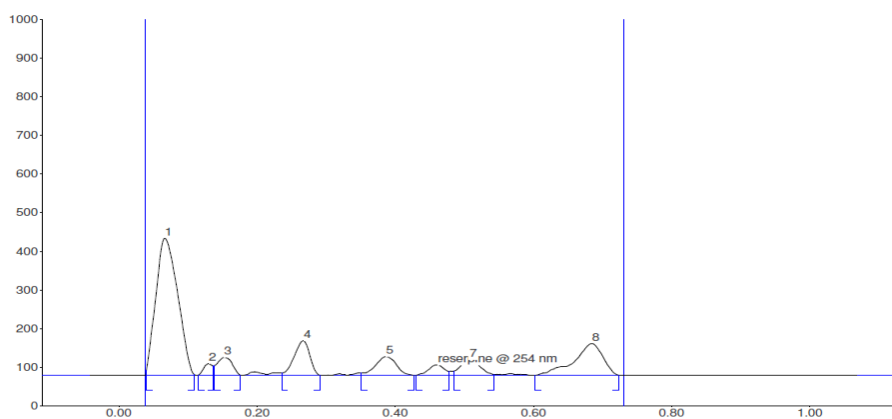
Track 9, ID: healthy 3

Fig.9:Chromatogram of healthy root sample 3

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	12.8	0.07	354.6	49.55	0.11	1.2	9304.0	52.78	unknown *
2	0.12	0.3	0.13	29.7	4.15	0.14	24.3	327.7	1.86	unknown *
3	0.14	24.9	0.15	46.1	6.45	0.18	0.3	830.2	4.71	unknown *
4	0.24	5.9	0.27	89.1	12.45	0.29	0.1	1666.0	9.45	unknown *
5	0.35	5.7	0.39	48.5	6.77	0.43	0.2	1205.1	6.84	unknown *
6	0.43	0.1	0.46	26.0	3.64	0.48	10.0	508.4	2.88	reserpine
7	0.49	10.8	0.51	39.8	5.57	0.54	2.2	934.0	5.30	unknown *
8	0.60	0.0	0.69	81.7	11.42	0.73	0.1	2851.8	16.18	unknown *

Table -9: Showing Peak area of healthy root sample 3.

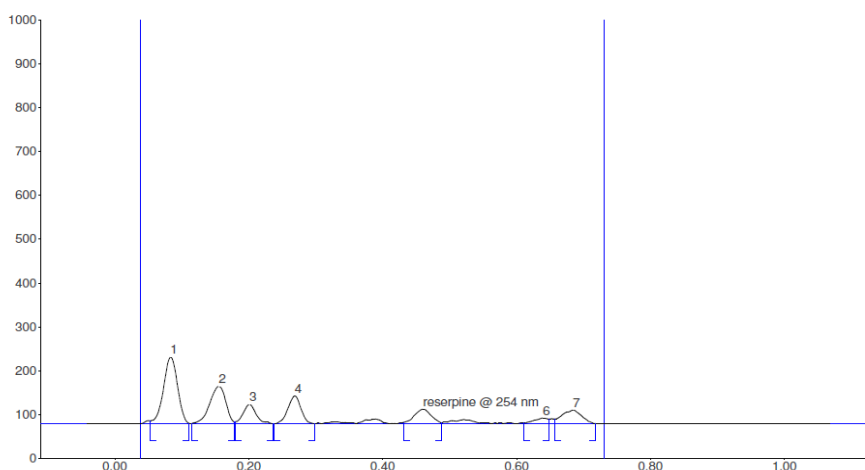Track 10, ID: infected 4

Fig. 10: Chromatogram of infected root sample 4.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.05	6.8	0.08	151.4	35.88	0.11	0.5	2668.3	34.30	unknown *
2	0.12	0.5	0.16	85.4	20.23	0.18	3.9	1701.4	21.87	unknown *
3	0.18	4.1	0.20	44.0	10.43	0.24	0.0	703.5	9.04	unknown *
4	0.24	0.1	0.27	63.8	15.11	0.30	0.3	1020.0	13.11	unknown *
5	0.43	3.0	0.46	33.2	7.88	0.49	2.8	698.3	8.98	reserpine
6	0.61	1.1	0.64	13.1	3.11	0.65	10.5	224.7	2.89	unknown *
7	0.66	10.7	0.69	31.1	7.36	0.72	0.4	763.7	9.82	unknown *

Table 10: Showing Peak area of infected root sample 4
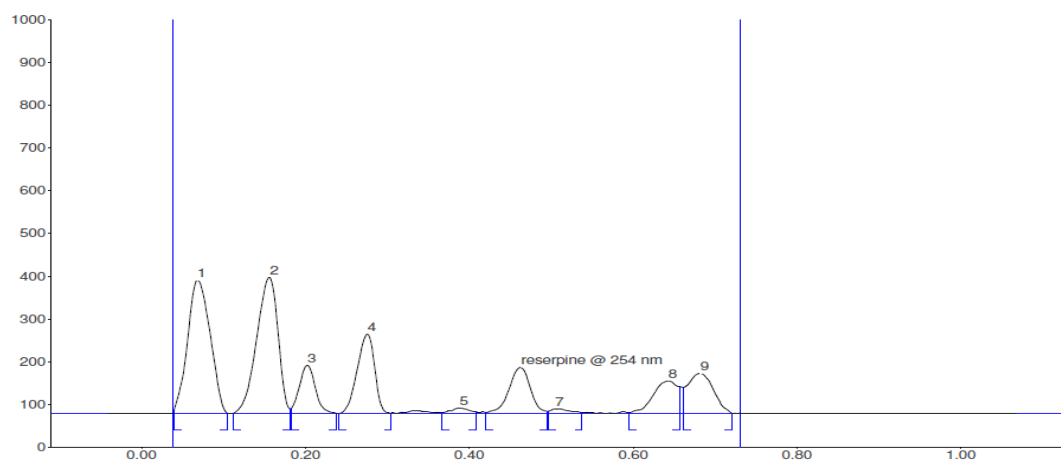
Track 11, ID: healthy 4

Fig. 11: Chromatogram of healthy root sample 4.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.04	8.7	0.07	310.9	25.36	0.11	0.2	7154.6	27.22	unknown *
2	0.11	0.3	0.16	317.7	25.91	0.18	10.4	6905.0	26.27	unknown *
3	0.18	11.6	0.20	112.7	9.20	0.24	0.5	1823.2	6.94	unknown *
4	0.24	0.0	0.28	185.6	15.14	0.31	1.0	3269.0	12.44	unknown *
5	0.37	1.9	0.39	12.9	1.05	0.41	3.4	227.6	0.87	unknown *
6	0.42	2.2	0.46	106.9	8.72	0.50	4.9	2330.0	8.87	reserpine
7	0.50	5.0	0.51	10.4	0.85	0.54	2.7	201.5	0.77	unknown *
8	0.60	1.1	0.64	75.7	6.18	0.66	62.6	1893.1	7.20	unknown *
9	0.66	60.6	0.68	93.1	7.60	0.72	0.3	2478.4	9.43	unknown *

Table -11: Showing Peak area of healthy root sample 4

**Spectrum scan**

Executed by	Anchrom Test Lab P. Ltd.
Mode	All detected peaks
Slit dimensions	6.00 x 0.45 mm, Micro
Optimize optical system	Resolution
Scanning speed	100 nm/s
Data resolution 1	0 nm/step
Reference spectrum, pos X	10.0 mm
Reference spectrum, pos Y	10.0 mm
Measurement Table	
Lamp	D2
Start wavelength	190 nm
End wavelength	400 nm
Measurement type	Remission
Measurement Mode	Absorption
Optical filter	Second order
Detector Mode	Automatic
Detector properties	
Y-position for 0 adjust	0.0 mm
Track # for 0 adjust	0
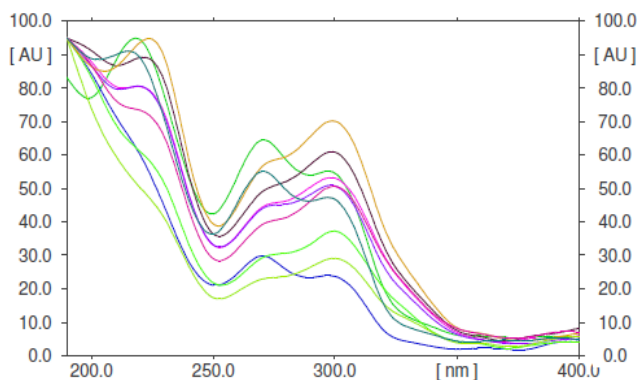
T	Rf	Substance	Max. @
1	0.46 Rf	reserpine	190 nm
2	0.46 Rf	reserpine	190 nm
3	0.46 Rf	reserpine	190 nm
4	0.46 Rf	reserpine	190 nm
5	0.46 Rf	reserpine	190 nm
6	0.46 Rf	reserpine	190 nm
7	0.46 Rf	reserpine	218 nm
8	0.46 Rf	reserpine	190 nm
10	0.46 Rf	reserpine	190 nm
11	0.46 Rf	reserpine	190 nm

Fig. 12: Reserpine on all tracks

Evaluation results

Evaluation Sequence

Track	Track type	Vial	Sample ID
1	Sam	1	infected 1
2	Sample	2	healthy 1
3	Sampl	3	infected 2
4	Sample	4	healthy 2
5	Sample	5	std reserpine
6	Sampl	5	std reserpine
7	Sample	5	std reserpine
8	Sample	6	infected 3
9	Sample	7	healthy 3
10	Sample	8	infected 4
11	Sample	9	healthy 4

Table of substances

		Position Tracks										
Substance	MD mm	1	2	3	4	5 6	7	8	9	0	1
reserpine	41.2	A	A	A	A	A A	A	A	A

Visualizer Document -   **Plate state Developed**

Image information - 366 nm - Image1

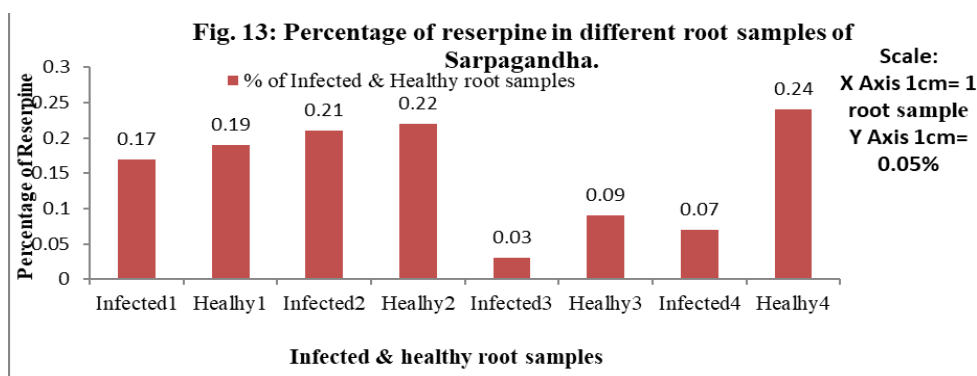|	
Illumination instrument	CAMAG Visualizer : 150503 (Visualizer_150503)
Digital camera type:	snr & Lens DXA252 : 306921208, Computar, 16 mm, f4.0
Created by:	on Anchrom Test Lab P.Ltd. :
Resolution	Full
Plate border size	-2 mm
Automatic capture	Off
Save mode	Lossy (JPG)
Exposure mode	Automatic, digital level: 85 %, Band
Capture settings:	
Image size:	1944 Pxl x 952 Pxl (0.10 mm/Pxl)
Exposure:	323.15 ms gain: 1.00
White balance	R: 1.40, G: 1.00, B: 1.20
Illumination type / correction type:	366 nm
Remission:	Individual correction
Display settings:	
White balance:	R: 1.00 G: 1.00 B: 1.00
Contrast enhancement:	1.00
Brightness:	0.00
Accentuation:	0.80
Color saturation:	1.30
Blank plate compensation:	N/A
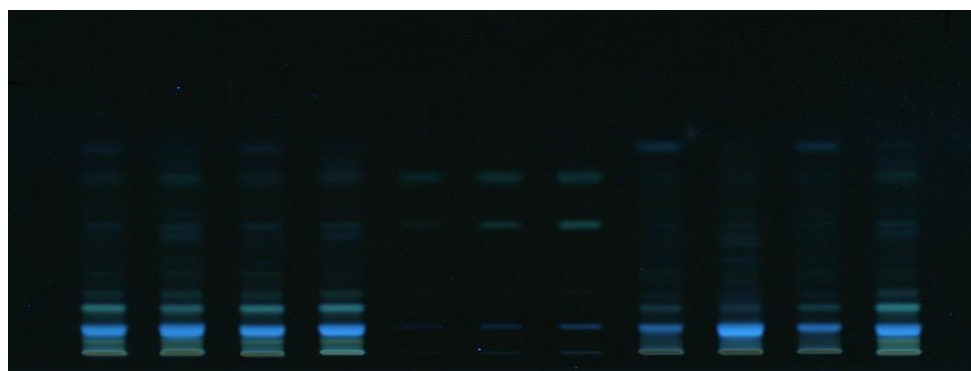
     IRS-1   HRS-1   IRS-2   HRS-2   Std -1   Std-2   Std-3   IRS-3   HRS-3   IRS-4   HRS-4

Image-1: Silica gel Chromatography Plate with sample marking at 366 nm wavelength showing different bands

Table 12: Percentage of reserpine in different root samples collected during rainy season in the month of August 2009**.**

Name of the sample	Sample peak area	Std. peak area	Sample dilution	Std. dilution	Percentage(%) of reserpine
Infected1	1755	4482	200	1	0.17
Healhy1	1498	4482	200	1	0.19
Infected2	1859	4482	200	1	0.21
Healhy2	1945	4482	200	1	0.22
Infected3	790	4482	200	1	0. 03
Healhy3	331	4482	200	1	0.09
Infected4	630	4482	200	1	0.07
Healhy4	2134	4482	200	1	0.24

Fig. 13: Percentage of reserpine in different root samples of Sarpagandha.

Scale:
X Axis 1cm= 1 root sample
Y Axis 1cm= 0.05%

RESULTS AND DISCUSSION

The reserpine on all tracks was shown in Fig.1. R F value of each track in maximum wavelength 366 was shown. Bands of reserpine were observed in full resolution illumination instrument camag visualize at in 366 nm as in Image-1. In all there are 11 bands. Band no. 1, 2, 3 and 4 were of infected 1, healthy 1, infected 2 and healthy2., band no. 5, 6 and 7 were of standard reserpine and 8, 9, 10 and 11 were of infected 3, healthy 3, infected 4 and healthy 4. The position of reserpine spot is variable in all standard and sample bands shows its variable amount. The percentage of reserpine was calculated with the help of peak area value.

The percentage of reserpine in infected and healthy root samples collected in the month of August 2009 was estimated by HPTLC method. . The percentage of reserpine in infected samples was 0.17, 0.21, 0.03 and 0.07. The percentage of reserpine in healthy samples shows 0.19, 0.22, 0.09 and 0.24. The percentage of reserpine was less in infected as compared to healthy samples as shown in Table-12 and graphical presentation in Fig.13. It is clear that the percentage of reserpine was decreased due to infection of root by *Macrophomina phaseolina*. Hence, the root rot disease is responsible for reducing the percentage of reserpine alkaloid content of *Rauwolfia serpentina* root.

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