Mahesh Dattani sensitizes the audience with the issue without being didactic and the audience is made to think of the condition of the hijras and their social identity. They are born alike all other human beings and separated pathetically from all others because of their gender issue. The dramatist has clearly portrayed the marginalized status and gender issue of hijras in the present play. They are deprived of all social equality and lingered behind from the mainstream of the society. To conclude, Mahesh Dattani's play Seven Steps Around the Fire raises a crucial issue of identity of hijras as the marginalized. The dramatist has explored that the bias against these people is even worse than any other. They are treated as marginalized human beings without voice, love and sympathy. Dattani portrays their status as unacceptable marginalized human in the modern society. Mahesh Dattani has revealed the social reality regarding the condition of these human beings.

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04

Estimation of Reserpine From Healthy and Diseased Roots of Rauwopfia serpentina (L.) Benth. ex. Kurtz (Sarpagandha) collected during rainy season in the month of December 2010 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 posses 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 December 2010 **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 pressure7 8, as tranquilizer7 8 etc. Many methods like UV spectroscopy, HPLC, HPTLC, gas chromatography, voltametry, polarography, room temperature phosphometry and spectrofluorimetry, 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, HPTLC method for detection of reserpine from Rauwolfia serpentina with validation data.

Materials 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 December 2010 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-5, IRS-5**, Medicinal plant garden, M.P.K.V., Rahuri, **HRS-6, IRS-6,** Nagarjun medicinal plant garden P. D. K. V., Akola **HRS-7, IRS-7** and S. G. M., Amravati University, Amravati **HRS-8, IRS-8** during rainy season in the month of December 2010 **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 ìl 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 chromatography plates were preliminarily washed with methanol and activated at 110 ^{0}c temperature for 5 minute in an oven. 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 6x0.45mì and scanned with speed 20 mm/sec. The monochromatic band width was set at 20mì. 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 Vin Cat s 4 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.

10 micro liter 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 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15. In the form of track 1 to 11.The Chromatogram shows tracks of reserpine present in samples and standard reserpine track. Peak area of reserpine was also shown in table 58, 59, 60, 61, 62, 63, 64, 65, 66, 67 and 68. The reserpine shows variation in peak area indicates variation in content in different samples. The first two chromatograms of each sample were infected and healthy roots.

The reserpine on all tracks was shown in Fig.1. Bands of reserpine were observed in full resolution illumination instrument camag visualize at as in 254 nm Image-1, 366 nm as in Image-2. In all there are 11 bands. Band no. 1, 2, 3 and 4 were of infected 5, healthy 5, infected 6 and healthy6., band no. 5, 6 and 7 were of standard reserpine and 8, 9, 10 and 11 were of infected 7, healthy 7, infected 8 and healthy8. 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 healthy and infected root samples collected in the month of December 2010 was estimated by HPTLC method. As compared to healthy and infected samples, the percentage of reserpine in healthy samples shows 0.15, 0.17, 0.15 and 0.17. The percentage of reserpine in infected samples was 0.09, 0.16, 0.12 and 0.15 as shown in Table-1 and Fig.1.The observations shown in table-1 indicate that reserpine percentage in infected samples is less than healthy samples. It is clear that the percentage of infected root of Rauwolfia serpentina was reduced due to fungal infection of Macrophomina phaseolina causing root rot disease. Hence, reserpine alkaloid content of Sarpagandha is decreased due to infection of Macrophomina phaseolina causing root rot disease.

Table- 1: Percentage of reserpine in different root samples collected in December 2010 estimated by HPTLC method.

Name of the sample	Sample peak area	Std. peak area	Sample dilution	Std. dilution	Percentage(%) of reserpine
Infected5	1755	4482	200	1	0.09
Healhy5	1498	4482	200	1	0.15
Infected6	1859	4482	200	1	0.16
Healhy6	1945	4482	200	1	0.17
Infected7	790	4482	200	1	0.12
Healhy7	331	4482	200	1	0.15
Infected8	630	4482	200	1	0.15
Healhy8	2134	4482	200	1	0.17

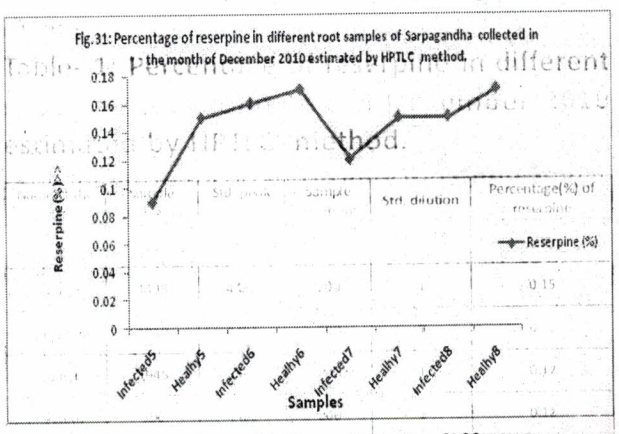


Fig1: Percentage of reserpine in different root samples of Sarpaghandha collected in the month of December 2010 estimated by HPTLC method

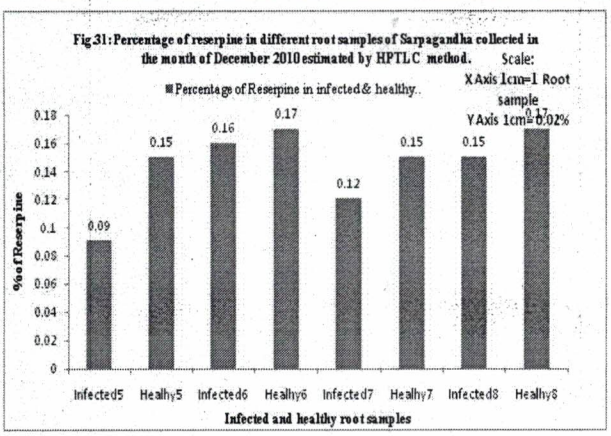


Fig1: Percentage of reserpine in different root samples of Sarpaghandha collected in the month of December 2010 estimated by HPTLC method

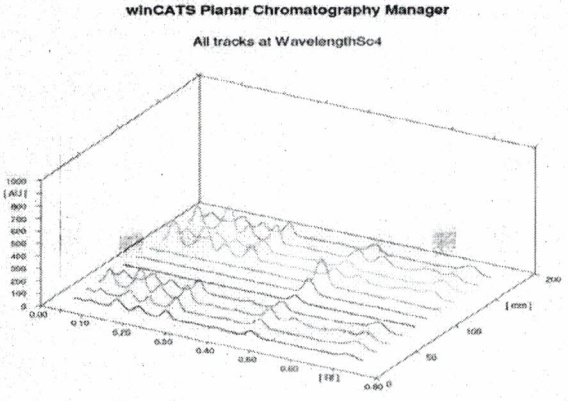


Fig.1: Three dimensional view of all reserpine tracks.

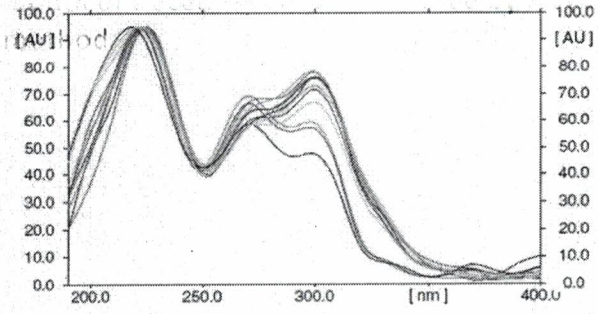
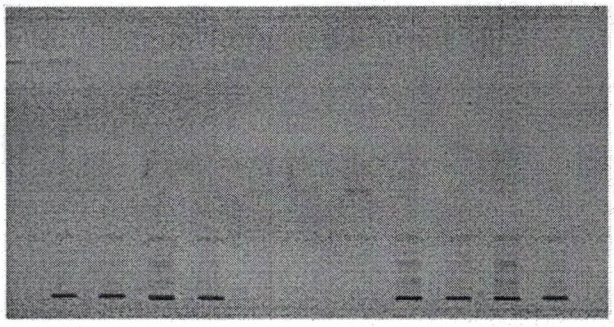
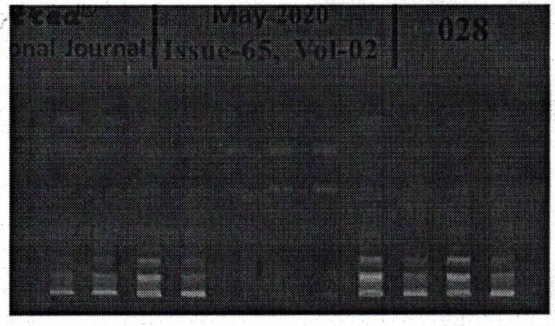


Fig. 1: Spectrum of reserpine on all Tracks.



IRS-5 HRS-5 IRS-6 HRS-6 Std-7 Std-8 Std-9 IRS-10 HRS-10 IRS-11 HRS-11

Image- 1: Silica gel Chromatography Plate with sample marking



IRS-5 HRS-5 IRS-6 HRS-6 Std-7 Std-8 Std-9 IRS-10 HRS-10 IRS-11 HRS-11

Image 2: Silica gel Chromatography Plate with sample marking at 366nm Wavelength showing different bands

Results and Discussion:

The reserpine on all tracks was shown in Fig.1. R-F value of each track in maximum wavelength 254 was shown. Bands of reserpine were observed in full resolution illumination instrument camag visualize at in 254 nm as in Image-1, 366 nm as in Image-2 and in white image-3. In all there are 11 bands. Band no. 1, 2, 3 and 4 were of infected 5, healthy5, infected6 and healthy6., band no. 5, 6 and 7 were of standard reserpine and 8, 9, 10 and 11 were of infected 7, healthy 7, infected 8 and healthy 8. 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 December 2010 was estimated by HPTLC method. . The percentage of reserpine in healthy samples was 0.15, 0.17, 0.15 and 0.17. The percentage of reserpine in infected samples was 0.09, 0.16, 0.12 and 0.15. The percentage of reserpine was less in infected as compared to healthy samples as shown in Table-1 and Fig.1. 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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