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Original Research Article

Estimation of Reserpine from healthy and diseased roots of sarpagantdha *Rauwopfia serpentina* L. Benth ex Kurz) by HPLC method

M M Dudhbhate*, B M Kareppa**

*Assistant Professor, Department of Botany, ACS College, Gangakhed, Maharashtra, INDIA.
**Department of Botany, DSM College, Parbhani-431401, Maharashtra, INDIA.
Email: mmdudhbhate@rediffmail.com, kareppabm@gmail.com

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 these fungi, Macrophomina phaseolina causes severe root rot disease. In order to Changes in reserpine from healthy and infected roots of Rauwopfia serpentine, healthy and infected roots were collected from medicinal plants garden, PDKV Akola and MPKV Rahuri. In the present study, estimation of reserpine from healthy and infected roots of Rauwopfia serpentina was carried out by HPLC method. It was observed that there is decrease in reserpine content in infected roots.

Key Words: Rauwolfia serpentina, Reserpine, HPLC, Macrophomina phaseolina.

***Address for Correspondence:**
Dr. M. M. Dudhbhate, Assistant Professor, Department of Botany, ACS College, Gangakhed, Maharashtra, INDIA.
Email: mmdudhbhate@rediffmail.com

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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 spectroscopy2, HPLC2, HPTLC2, gas chromatography5, voltametry5, polarography5, room temperature phosphometry5 and spectrofluorimetry5, 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.

MATERIALS AND METHODS

Collection of Plant material and estimation of reserpine: The plants of *Rauwolfia serpentina* L. Benth ex Kurz were collected from medicinal plants garden, PDKV Akola and MPKV Rahuri (Maharashtra). Standard Reserpine was purchased from Natural Remedies Pvt. Ltd. Bangalore and estimation of reserpine from healthy and infected roots was carried out at Radiant Research Services Pvt.Ltd. Bangalore.

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Experimental Conditions:

Sample Description : *Rauwolfia serpentina* root extracts
Type of Instrument : LC
Gradient : High Pressure
Detector : UV
Wavelength (nm) : 268
Model No : VARIAN
Column Part No. : 297045
Sample code : Reserpine
MOBILE PHASE : Methanol: 0.005M NH4cl at pH 5: 50:50
Flow Rate : 1.5mL/min
Injection Volume : 20 µl
Column Dimension ID : 250X4.6 mm, 5µ
Diluents : Methanol
Quantification : Area/Area%

Standard preparation: 3 mg of each standard was weighed and dissolved in 1 ml of solvent, from which 100 µl was taken and made up to 1 ml with solvent, from this stock solution 200 µl was injected.

Sample preparation: Roots were excised from the plants, washed with running tap water. It was dried in an oven at 60 ^{0}C and made into fine powder.10 mg of each sample was weighed separately and dissolved in 10ml methanol.

RESULTS AND DISCUSSION

The content of reserpine in these four samples was evaluated by HPLC method. Reserpine shows a peak at 3.473 min retention time. HPLC Chromatogram of all the samples has shown four major peaks i.e., 3.282, 3.248, 3.307and 3.123 respective min. retention time. The percentage of reserpine was 4.54, 3.24, 1.38 and 1.75 from healthy and infected samples as compared to standard as mentioned in table.1 and fig. I, II, III, IV and V.

Calculation

Sample area Standard dilution
Percentage of = ---------- x ---------- x100
Reserpine Standard areas Sample dilution

Table 1: Estimation of Reserpine from *Rauwolfia serpentina* root by HPLC method

Sample name	RS healthy(2)	RS infected(2)	RS healthy(1)	RS infected(1)
sample area	1904911.25	1361730.125	580324.75	737015.188
standard area	4192247.75	4192247.75	4192247.75	4192247.75
sample dilution	1	1	1	1
standard dilution	0.1	0.1	0.1	0.1
% Of Reserpine Standard	4.54	3.24	1.38	1.75

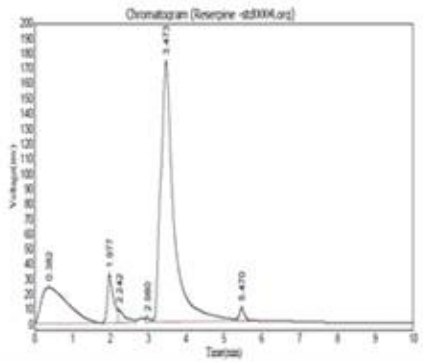

Figure 1

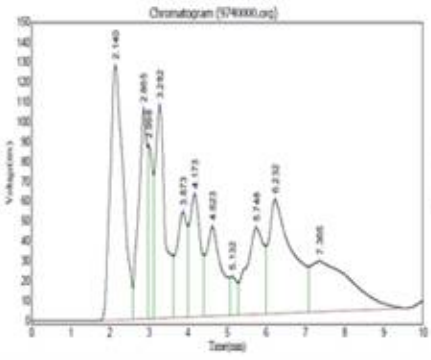

Figure 2

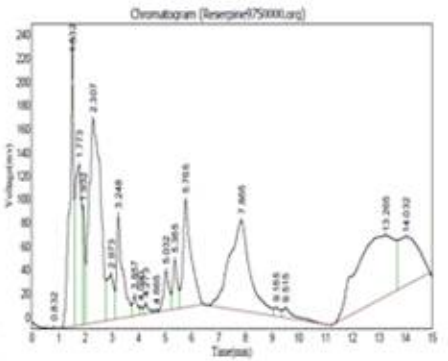

Figure 3

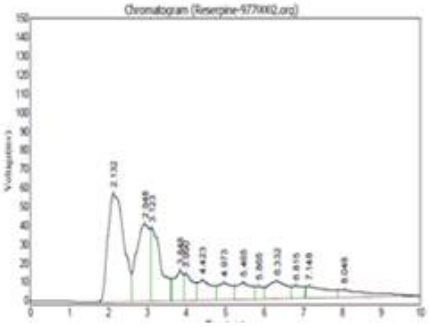

Figure 4

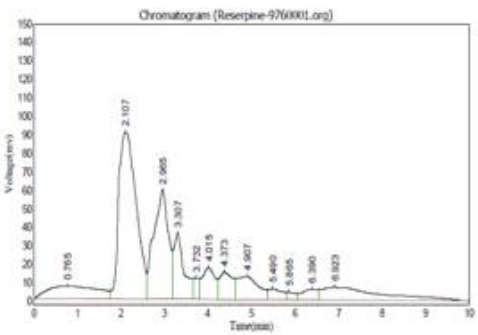

Figure 5

Figure 1: HPLC chromatogram of Reserpine Reference standard at 268 nm; **Figure 2:** HPLC chromatogram of methanolic extract of healthy plant roots collected from Rahuri at 268nm; **Figure 3:** HPLC chromatogram of methanolic extract of infected plant roots collected from Rahuri at 268nm; **Figure 4:** HPLC chromatogram of methanolic extract of infected plant roots collected from Akola at 268nm; **Figure 5:** HPLC chromatogram of methanolic extract of healthy plant roots collected from Akola at 268nm.

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