International Journal of Pharma and Bio Sciences

RESERPINE CONTENT OF *RAUWOLFIA SERPENTINA* IN RESPONSE TO GEOGRAPHICAL VARIATION

HAREESH KUMAR V[*1], NIRMALA[2], SHASHIDHARA S[1] AND RAJENDRA C E[3]

[1]Department of Pharmacognosy, Government College of Pharmacy, Bangalore, India – 560027.
[2]Department of Pharmaceutics, Government College of Pharmacy, Bangalore, India - 560027
[3]Drug Testing Laboratory, Drug Control Department, Bangalore, India.

Corresponding Author hareesh_shree86@yahoo.co.ln

ABSTRACT

Important requirement in the evaluation of herbal drug include the estimation of active constituent. Different factors like climate, altitude, rainfall and other conditions responsible for growth of plants may affect the content of active constituents. Collection of drug from different geographical sources can give useful conditions required for the production of maximum amount of secondary cell constituents. *Rauwolfia* Samples were collected from four different parts of southern India. HPLC chromatogram was developed for standard reserpine. Different samples were extracted using methanol and extracts were subjected to HPLC analysis to find out the content of Reserpine for preliminary information about the conditions that may influence on production of active constituents. Significant variation in the content of reserpine has been recorded.

KEY WORDS

Reserpine, *Rauwolfia serpentina,* HPLC, Geographical regions.

INTRODUCTION

The increasing demand for herbal medicines both in the developing and developed countries inevitably led to maintaining the quality and purity of the herbal raw materials and finished products[1].

Number of factors relating to climate, altitude, rainfall and other conditions responsible for growth of plants affect the quality of Herbal ingredients present in a particular species even when it is produced in the same country. These conditions may produce major variations in the active ingredients present in plants[1,3].

Several theories have been proposed to explain potential tradeoffs between growth and secondary metabolite production. The resource availability theory suggests that the way a plant defends itself ultimately depends on resource availability and its intrinsic growth rate. This theory predicts that the rapidly growing plants in resource rich habitats contain low levels of highly mobile secondary metabolites (Alkaloids and Cyanogenetic glycosides). Nitrogen is taken up early in the growing season in excess of the plant's need for growth. Excess nitrogen is available to be synthesized into N – based secondary metabolites[2].

Differences in growth trajectories of plant may also lead to both positive and negative correlations between intrinsic growth rate and the ability to compensate for herbivory.

Understanding how environmental factors affect the production of secondary metabolites will be of great importance for the conservation of medicinal plants and optimizing field growth conditions for maximal recovery of phytomedicinal chemicals[2].

The aim of the present study is to get preliminary information regarding the evaluation of active constituent reserpine for samples collected from different geographical sources to ascertain whether there is uniformity or variation exists. Also to find out which of the samples has maximum or minimum active constituent by HPLC studies.

MATERIALS AND METHODS

Collection of Plant material and Standard:

The plants of *Rauwolfia serpentina* L. Benth ex Kurz were collected from Trissur, (Kerala), Shimoga (Karnataka), Coimbatore (Tamil Nadu), Tirupathi (Andhra Pradesh). They were identified and authenticated by Dr. Jawahar Raveendra, Botanist, Foundation for Revitalization of Local Health Tradition (FRLHT), Bangalore, Karnataka. (Climatic conditions of the above geographical regions are given in table – 1).

Table – 1

Geographical Location and Climatic Conditions of different accessions.

Sl.no	Name of accession	Co ordinates	Altitude (metres above mean sea level)	Temperature In ^{0}C	Average rainfall per annum (mm)
1	Shimoga	latitudes 13°27' and 14°39' N and between the longitudes 74°38' and 76°04' E	640	26-35	900
2	Thirupathi	latitude 13° and 14° N and longitude 17° E	1066.66	26-42	300
3	Trissur	10^0 32 N and 76^0 15 E.	52.3	22.5-32.3	3,159
4	Coimbatore	Latitude 11°1′6″N 76°58′21″E longitude 11.01833°N 76.9725°E	409	12-38	700

Reference standard of Reserpine was purchased from Natural Remedies Pvt. Ltd. Bangalore.

All the solvents and chemicals used for extraction and analysis were of high purity and quality.

Extraction

The roots were excised from the plants, washed with running tap water and dried in an oven at a temperature of not more than 60^0C. Dried roots were coarsely powdered and subjected for extraction. 100 mg of the powder from each plant was separately treated with ammonia solution and then extracted with methanol until the extracts were colorless. The methanolic extracts of each plant were combined separately and concentrated in a rotary evaporator, and dried in a vacuum oven[3].

HPLC analysis:

Sample preparation:

The extract of each plant was transferred to 5ml volumetric flasks separately with the help of methanol (HPLC grade), sonicated for few minutes filtered and volume was made upto mark with methanol.

Standard preparation:

0.004% of Reserpine in Methanol was prepared.

Instrument:

Schimadzu LC – 20, equipped with auto sampler and LC SOLUTIONS software.

Experimental conditions:

Column : Lichrosorb C – 18 (25 X 0.5cm 10µ)

Mobile phase : Acetonitrile: Buffer (Prepared by weighing 6.80 g of potassium dihydrogen phosphate in 1000ml HPLC water

and pH was adjusted to 3.0 with Orthophosphoric acid) 35: 65
Volume injected : 20µL
Flow rate : 1ml/min
Detection wavelength : 268nm
Temperature : Ambient temperature
Method used : Bracketing method with the triplicate and duplicate injections of standard and sample respectively.

RESULTS AND DISCUSSION

The content of reserpine in different samples was evaluated by HPLC studies. Reserpine has shown a peak at 16.596min retention time. Extract of *Rauwolfia* plant collected from diferent locations namely Trissur(Kerala), Shimoga(Karnataka), Coimbatore(Tamil Nadu) and Tirupathi(Andhra Pradesh) was used for the analysis. HPLC Chromatogram of all the samples has shown five major peaks including reserpine. Sample collected from Coimbatore has shown maximum amount amount of reserpine of 0.1442%. where as sample collected from Shimoga has shown minimum amount of reserpine of 0.0382%. variation in the content of reserpine range from 0.0382 to 0.1442% (Table – 2 and figure – 1) hence there is a need to define the conditions required for production of maximum amount of reserpine by studying all the parameters individually.

Table – 2
Reserpine concentration in Rauwolfia collected from different Geographical Source.

Sl. No.	Plant Collected From	Retension time (min)	Area under the curve	Figure no.	Concentration of Reserpine in %
1	Shimoga	16.544	211322	4	0.0382
2	Trissur	16.575	608778	6	0.0947
3	Tirupathi	16.662	397061	5	0.0707
4	Coimbatore	16.491	699626	7	0.1442

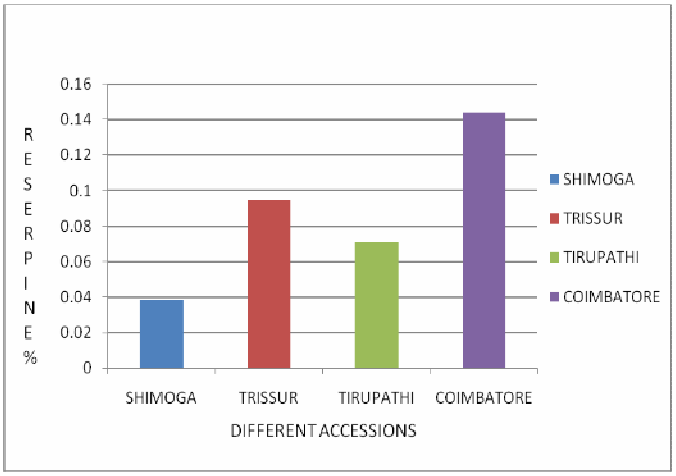


Fig – 1
Graph showing concentration of Reserpine in Rauwolfia serpentina collected from different accessions

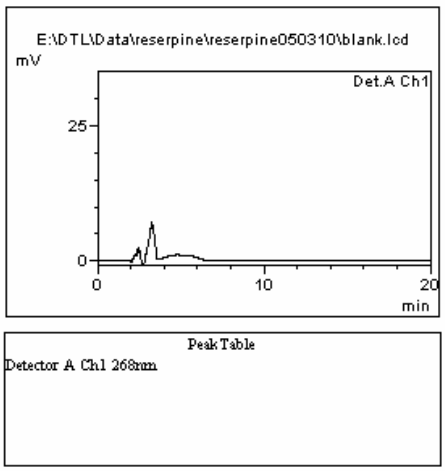


Fig – 2

HPLC chromatogram of methanol (blank) at 268 nm

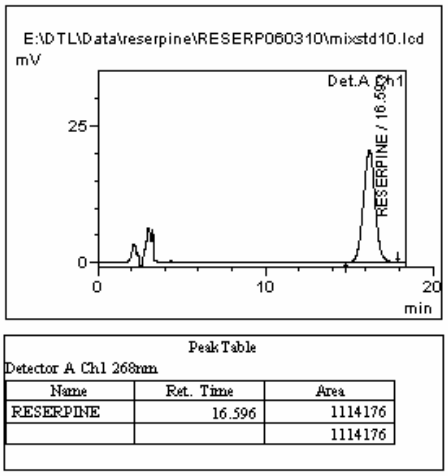


Fig – 3

HPLC chromatogram of Reserpine Reference standard at 268 nm

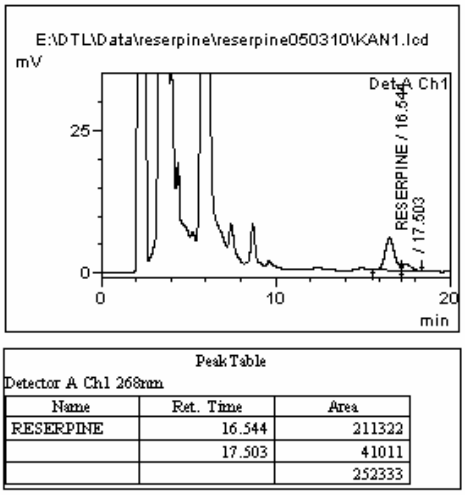
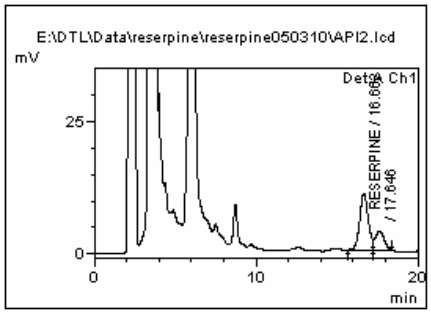


Fig – 4

HPLC chromatogram of methanolic extract of plant collected from Shimoga at 268nm.



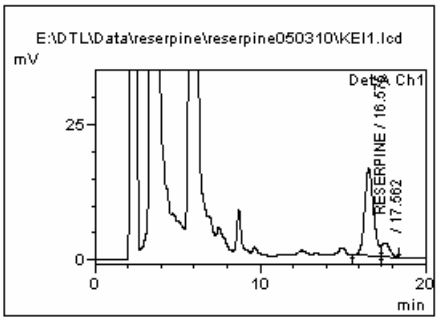
Peak Table

Detector A Ch1 268nm

Name	Ret. Time	Area
RESERPINE	16.662	397061
	17.646	134987
		532048

Fig – 5

HPLC chromatogram of methanolic extract of plant collected from Tirupathi at 268nm



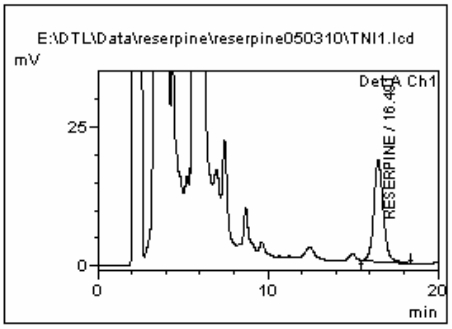
Peak Table

Detector A Ch1 268nm

Name	Ret. Time	Area
RESERPINE	16.575	608778
	17.562	87415
		696193

Fig – 6

HPLC chromatogram of methanolic extract of plant collected from Trissur at 268nm



Peak Table

Detector A Ch1 268nm

Name	Ret. Time	Area
RESERPINE	16.491	699626
		699626

Fig – 7

HPLC chromatogram of methanolic extract of plant collected from Coimbatore at 268nm

ACKNOWLEDGEMENTS

Authors are very thankful to all the members of American Association of Government College of Phamacy Alumni New York USA, Dr. P E Rajasekharan Senior Scientist, Indian Institute of Horticultural Research, Hesaraghatta, Bangalore and Mr. Jagannathan, medicinal plant cultivator, Chungarangulam, Kerala for their help and support.

REFERENCES

1. Pulak K Mukherjee. Quality Control of Herbal Drugs, 1st edition, Business Horizons: 120-125, (2002)
2. Cai ZQ, Wang WH, Yang J, Cai CT., Growth, Photosynthesis and root reserpine concentrations of two Rauwolfia species in response to light gradient. Industrial Crops and Products, 30: 220-226, (2009)
3. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 26th edition, Nirali Prakashan: 466-470, (2004)
4. Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis A Thin Layer Chromatography Atlas, Springer Verlag: 70-71, (1984)
5. WHO monographs on selected medicinal plants, Vol. I, World Health Organization: 221-230, (1999)
6. Indian Herbal Pharmacopoeia, Revised edition, Indian Drug Manufacturers Association: 345-354, (2002)
7. Monograph number 9447. Merck Index. 12th Edition (Electronic version), 1999 Merck & Co., Inc., Whitehouse Station, NJ, USA.
8. Qureshi SA, Nawaz A, Udani SK, Anmi B., Hypoglycaemic and Hypolipidemic Activities of *Rauwolfia serpentina* in Alloxan-Induced Diabetic Rats. International journal of Pharmacology: 1-4, (2009)
9. Sunday O Idowu, Olagire A Adegoke, Ajibola A Olaniyi., Improved Colorimetric Determination of Reserpine in Tablets Using 4-Caboxyl-2,6-dinitrobenzene diazonium ion (CDNBD). Tropical Journal of Pharmaceutical Research, 6(2): 695-703, (2007)
10. Sameer Agarwal, Narayana BDA, Poonam Raghuvanshi, Srinivas KS., Quantitative Detection of β-Asarone in Acorus calamus using HPTLC. Indian Drugs, 32(6): 254 - 257, (1994)
11. Viel C, Galand N, Pothier J, Dollet J, OPLC and AMD., Recent techniques of planar chromatography: Their interest for separation and characterization of extractive and synthetic compounds, Fitoterapia: 2-14, (2002)
12. Dhruv K Singh, Bhavana Srivastava, Archana Sahu., Spectrophotometric Determination of Rauwolfia Alkaloids: Estimation of Reserpine in Pharmaceuticals. Analytical Sciences, 20: 571-573, (2004)