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PHYTOCHEMICAL ANALYSIS OF ROOTS AMONG VARIOUS GENOTYPES ACROSS DIFFERENT AGE GROUPS IN *RAUWOLFIA SERPENTINA* (L.) BENTH BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Rauwolfia serpentina contains 50 indole alkaloids and most of the total alkaloid content is present mainly in root bark. For centuries, roots of *Rauwolfia serpentina* have been used in traditional Unani and Ayurvedic medicine as a valuable remedy against many complex diseases. The present investigation was carried out in relevance to isolation of bioactive constituents from the roots amongst diverse genotypes across different age groups of *Rauwolfia serpentina* by using High Performance Liquid Chromatography analysis. The peaks were identified using reference standard of reserpine and the content of reserpine in methanolic root extract was determined using area under the curve of sample and reference standard. The combination pertaining genotype SRS 1 at 18 months was found significantly superior in recording the highest reserpine content (3.039 ± 0.03%) whereas, the lowest was noticed in genotype SRS 3 (1.818 ± 0.09%) for the same age group. The High Performance Liquid Chromatography evaluation illustrated that the principal bioactive component is reserpine. Root biomass along with the formation of new adventitious roots has a very direct effect in the per cent reserpine content of the roots. This is the foremost attempt to reveal the High Performance Liquid Chromatography analysis of roots amid various genotypes across different age groups of *Rauwolfia serpentina*.

KEYWORDS: ***Rauwolfia serpentina*, roots, genotypes, age, High Performance Liquid Chromatography, reserpine.**

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INTRODUCTION

Rauwolfia serpentina is an erect and medicinally important perennial shrub which belongs to the family Apocynaceae. It is commonly known as Sarpagandha, Chandrabhaga, Sutranbhi and Serpentwood. The plant is indigenous to Bangladesh, India and other tropical region of the world.[1] Roots grow nearly vertical, tapering up to 15 cm thick at the crown and long giving a serpent-like appearance, occasionally branched or tortuous developing small fibrous roots. Among the 5 different species of *Rauwolfia, R. serpentina* is preferred for cultivation because of higher reserpine content in the root. *Rauwolfia serpentina* contains 50 indole alkaloids and most of the total alkaloid content is present mainly in root bark.[2] The principal hypotensive alkaloid was identified as reserpine. Other includes a number of indole alkaloids: yohimbine type of derivatives *viz.*, deserpidine, yohimbine, corynantheine; heteroyohimbane derivatives *viz.,* ajmalicine, reserpiline, alstonine and dihydroindole derivatives *viz.,* ajmaline. Among all the alkaloids reserpine, yohimbine, serpentine, deserpidine, ajmaline, *etc.* are used to treat hypertension.[3] Green plants of *Rauwolfia serpentina* synthesize and preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations.[4] Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds.[5] Hence, the present study was carried out to explore the presence of highly bioactive constituents in the roots of different genotypes under various age groups of *Rauwolfia serpentina* by imparting an approach through High performance liquid chromatography analysis.

MATERIALS AND METHODS

The study in relevance to Phytochemical analysis of roots amid various genotypes across different age groups of *Rauwolfia serpentina* was undertaken during the year July 2011 at Indian Institute of Integrative Medicine, Jammu, Jammu and Kashmir, India. During the year 2010-2011 in the Poly house of College of Forestry, Sirsi, Uttar Kanada District, Karnataka totally five genotypes from the germplasm bank which was maintained under the National Medicinal Plant Board Project were used for analysis and they include 2 from coastal region (KMT 1 and KMT 2) originating from high rainfall coastal zone with an altitude less than 50 meter above mean sea level and three genotypes from upghat region of Sirsi (SRS 1, SRS 2 and SRS 3) originating from moderately high rainfall area with an altitude of 630 meter above mean sea level. The design followed under this research was Completely Randomized Design (C.R.D) with 3 replication each consisting of 15 plants for every harvest. Poly vinyl chloride (PVC) pipes of 45 cm length and diameter of 4 inch were filled with soil, sand and FYM in the ratio of 2:1:1 as per standard [6] and plants amid diverse genotypes of *Rauwolfia serpentina* were monitored for 6 months. The Reference standard of reserpine which was used under this study was purchased from Sigma-Aldrich Pvt. Ltd. New Delhi. The entire solvents and the chemicals which were used under this investigation were of high grade.

Extraction

The roots were excised from different genotypes of *Rauwolfia serpentina.* Roots were washed under the running tap water which is then subjected for drying in an oven at a temperature not more than 60°C. The dried root material was coarsely powdered and 100 mg of the powdered root material from each sample was made slurry in ammonia solution and kept for 2 hours and then extracted with l0 ml of methanol at a temperature of 60°C on a water bath for about 15min. This extract was finally filtered and concentrated under the reduced pressure. For removing the remaining solvent extract was transferred into the Petri-dish and heated over a water bath. The extract was dried in vacuum oven at a temperature of 60°C to a constant weight to obtain residue. This residue was lastly dissolved in 1 ml of methanol and it was used for extraction.[7]

Preparation of sample solution

Sample solution was prepared as per the method described under extraction. Final volume was made up to 5ml using methanol in volumetric flask.

Preparation of standard solution

One mg of standard reserpine was dissolved in 25 ml of methanol and sonicated to get the concentration of 0.004% w/v.

Chromatographic conditions

Column: Lichosorb C-18 (4.6 x 250 mm)
Mobile phase: Acetonitrile: Buffer 35: 65
Detection wavelength: 268 nm
Flow rate: 1.0 ml/min

Procedure

20µl of both standard and sample solution were injected in High Performance Liquid Chromatography and recorded the chromatogram. Reserpine peak was identified using reference standard peak obtained by same procedure. The content of reserpine in *Rauwolfia serpentina* root extract was calculated using area under the curve of standard and sample peaks.

RESULTS

High Performance Liquid Chromatography involves the qualitative and quantitative determination of active constituents present in the plant extracts.[8] High Performance Liquid Chromatography analysis was carried out by using reserpine as standard substance and methanolic extract of *Rauwolfia serpentina* prepaid by the method of Wagner as sample. High Performance Liquid Chromatography analysis worked out effectively in order to determine the per cent (%) content of reserpine in the methanolic extract of roots of *Rauwolfia serpentina* across different age groups.The reserpine content was significantly influenced due to the genotypes at different growth stages. Among 5 different genotypes used for analysis of reserpine content subsequently from roots of *Rauwolfia serpentina* at different time period revealed that genotype SRS 1 pertained high evacuation of reserpine (1.818%). The least reserpine content was noticed in genotype SRS 3 (1.044%) during experimentation. The percent increase recorded was 42.57%.Per cent reserpine content in the

roots varied significantly due to the age of seedlings. Along with this, 18 months old seedlings recorded significantly higher reserpine content (2.242%) and lowest was detected in 12 months old seedlings (0.552%). Initially the accumulation of reserpine in roots was slow and rapid increase was noticed between 15 and 18 months growth period. The per cent increase was 57.95% between 12 to 15 months old seedlings compared to 41.43% between 15 to 18 months old seedlings irrespective of genotypes.The interaction between genotypes and age of the seedlings exhibited significant variation on per cent reserpine content during experimentation. The treatment combination pertaining seedling of genotype SRS 1 with 18 months was found significantly superior in recording the highest reserpine content (3.039 ± 0.03%) whereas, the lowest was noticed in the seedlings of genotype SRS 3 (1.818 ± 0.09%) for the same age. Among the 12 month age group all the genotypes were on par with each other except the genotype SRS 2 which recorded highest reserpine content (0.881%).

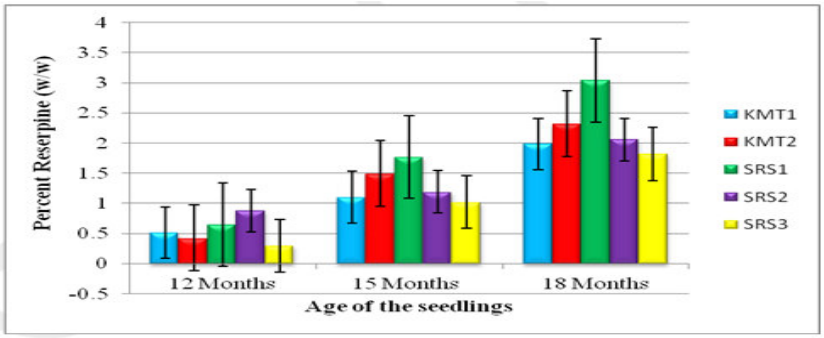
Table 1

Influence of genotype, age of plant and their interaction on percent reserpine content in roots of Rauwolfia serpentina

Genotype	Percent reserpine content			Mean (%)
	12 months	15 months	18 months	
KMT 1	0.514 ± 0.02	1.100 ± 0.03	1.980 ± 0.07	**1.198**
KMT 2	0.426 ± 0.03	1.491 ± 0.04	2.317 ± 0.07	**1.411**
SRS 1	0.647 ± 0.09	1.769 ± 0.09	3.039 ± 0.03	**1.818**
SRS 2	0.881 ± 0.06	1.188 ± 0.01	2.055 ± 0.06	**1.374**
SRS 3	0.295 ± 0.02	1.020 ± 0.04	1.818 ± 0.09	**1.044**
Mean	**0.552**	**1.313**	**2.242**	**1.369**

Graph 1

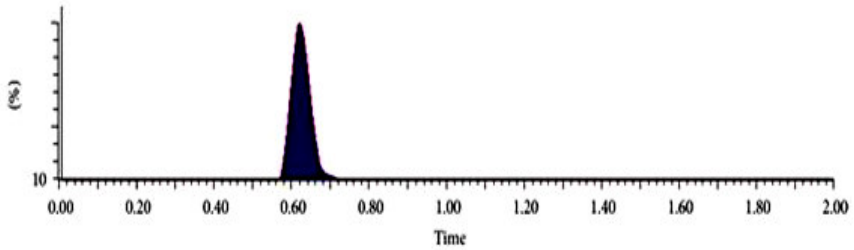
Variation for percent (%) reserpine content of roots in five genotypes of Rauwolfia serpentina across different age groups



Coefficient of Variation: 4.43% , Genotypes: SEm = 0.402 CD = 0.83 at P level < 0.05 , Age: SEm = 0.391 CD = 1.21 at P level < 0.05 Interaction: SEm= 0.303 CD = 0.718 at P level < 0.05

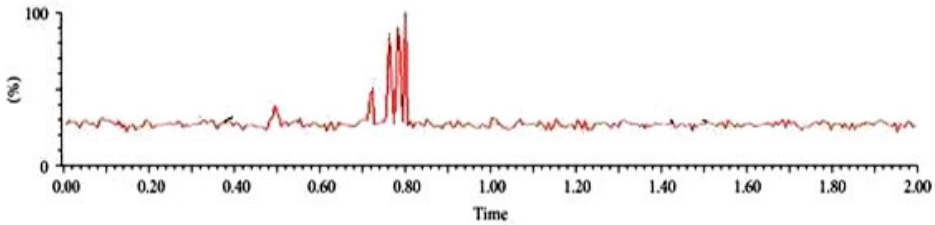
Graph 2

HPLC chromatogram of methanol (blank) at 268 nm

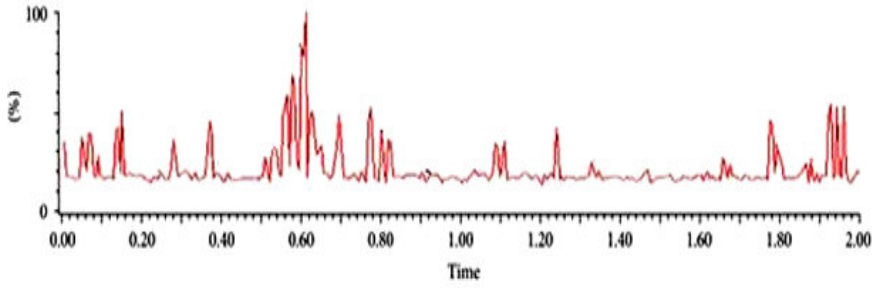


Graph 3

HPLC chromatogram of methanolic extract of plants of five genotypes of Rauwolfia serpentina at 12 month age



Graph 4
HPLC chromatogram of methanolic extract of plants of five genotypes of Rauwolfia serpentina at 15 month age



Graph 5
***HPLC chromatogram of methanolic extract of plants of five genotypes of Rauwolfia serpentina at 18 month* age**

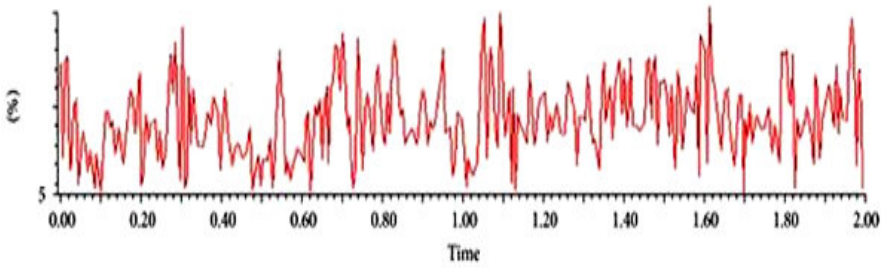


Figure 1
Lyophilisation of root extract of Rauwolfia serpentine

Figure 2
Methanolic extract of root of Rauwolfia serpentina

DISCUSSION

Genotype KMT 1 engulfs low amount of reserpine content. This may be due to low plant height plant produces low root biomass which results in low accumulation of reserpine. The genotype KMT 2 although pertained low root length but the per cent reserpine content in genotype KMT 2 was next to the top genotype SRS 1. This may be due to rigid root biomass excellent synthesis of reserpine from roots will take place. Moreover, our results are on par with the results of Kirillova *et al*. [9] who had achieved sequential isolation of enzyme superoxide dismutase from a tissue of *Rauwolfia serpentina*. These results also relates with the findings of Riad *et al.* [10] in traditional medicinal plants. Genotype SRS 1 engulfed high root length with immense root biomass for all three growth stages which leads to high perpetuity of reserpine content in roots. Formation of new adventitious roots. had a very direct effect in the percent reserpine content in the roots of *Rauwolfia serpentina.* Similar to our results, Bhavsar *et al*. [11] revealed the variation in the pharrnacognotic characters in adventitious roots of *Adathoda vasica.* Our results are in equivalence to the observations recorded by Edgar *et al.* [12] in amino acid determination from medicinal plants and also by Vivek [13] in quantification of podophyllotoxin among various populations of Indian Mayapple. At the age of 12 months, genotype SRS 2 possessed very good plant height & engulfed high reserpine content in the roots. But at the age of 18 months, reserpine content of genotype SRS 2 got reduced. This may be due an abrupt increase in the reproductive phase of genotype SRS 2 which leads to high onset of inflorescence and subsequently low reserpine accumulation in roots due to induction of low enzymes activity *viz.* superoxide dismutase. These results are in conformity with the findings of Srivastava *et al*. [14] in citronella. Subsequently Dennis *et al.* [15] revealed same conclusion on adaptaion in *Astragalus membranaceus* and also Fai *et al.* [16] putforth similar findings regarding antibacterial activities of medicinal plants. Moreover Hastati *et al.* [17] illustrate parallel findings in relevance to determination of curcumin pigment. Genotype SRS 3 pertained low reserpine content subsequently for all three growth stages as compared with other genotypes. This may be due to formation of low shoot length resulting in very low plant height and low root biomass. Similar to our results, Serraj *et al*. [18] recorded same observation in *Withania somnifera* and also Wang *et al.* [19] conformed matching results regarding Sedum medicinal plants. Furthermore our results correlate with the verdict of Pfoze *et al.* [20] in *Mahonia manipurensis* and of Daisy *et al.* [21] in *Curcurbita maxima*

CONCLUSION

It is clearly revealed that per cent reserpine content in the roots of *Rauwolfia serpentina* had positive interaction with the age of the plant. Genotype, tissue type and the development stage may be all determining factors in the comparative ability of roots to respond to the accumulation of reserpine. Moreover root biomass along with the formation of new adventitious roots has a very direct effect in the per cent reserpine content in the roots of *Rauwolfia serpentina.* Our systematic investigation divulges that higher reserpine content was noticed in genotype where formations of new adventitious roots take place.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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