

Full Length Research Paper

## Quantification of reserpine content and antibacterial activity of *Rauvolfia serpentina* (L.) Benth. ex Kurz

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Reserpine is well known bioactive compound isolated from *Rauvolfia serpentina*. The aim of this study was to quantify reserpine content and evaluate the antibacterial activity of methanol extracts of *R. serpentina* against *Salmonella typhimurium*, *Escherichia coli*, *Citrobacter freundii*, *Proteus vulgaris*, *Enterococcus faecalis* and *Staphylococcus aureus*. Roots of *R. serpentina* were collected from Gadarpur and Uttarakhshi of Uttarakhand State, India. The antibacterial activity of the methanol extracts was evaluated by determination of minimum inhibitory concentration (MIC) and the diameter of zone of inhibition (ZOI) against both Gram positive and Gram negative bacteria using agar well diffusion method. The study reveals that reserpine content was higher (0.37%) in the sample collected from Gadarpur, whereas it was found to be 0.31% in sample collected from Uttarakhshi. The highest zone of inhibition (13 mm) with lowest MIC (625 µg) was observed against *Staphylococcus aureus* and highest MIC (10 mg) was observed against *Escherichia coli*, whereas *Proteus vulgaris* was observed resistant to tested extracts upto 10 mg. *R. serpentina* contain good amount of reserpine and exhibited strong antibacterial activity against most of the tested human pathogenic bacteria. Therefore, the results of the study support the folklore claim of the plant species.

**Key word:** High performance thin layer chromatography (HPTLC), reserpine, ciprofloxacin, zone of inhibition, minimum inhibitory concentration.

### INTRODUCTION

Many bacteria and fungi produce human diseases which are currently controlled through the massive use of synthetic bactericides and fungicides. Some of them are resistance to synthetic drugs and caused therapeutic problem (Guillemot, 1999). Plants extracts are one of the options that have recently received attention and expected that it will be active against synthetic drug resistant pathogens. Therefore, the search for plant based new antibacterial and antifungal agents are imperative. *Rauvolfia serpentina* extract have been used to treat infections for thousands of years in Indian system

of medicines. It is used for the treatment of fever, anxiety, epilepsy, snake bite, rheumatism, insanity, eczema, intestinal disorders, psychiatric disorders, nervous disorders, cardiovascular disorder, bacterial infections and in the management of hypertension schizophrenia (Kirtikar and Basu, 1993; Gaur, 1999; Joshi and Kumar, 2000; Manuchair, 2002).

Reserpine has highly complex pattern of activity and is the main biological active phytochemical of the commercial drug Sarpagandha prepared from *R. serpentina*. Indole alkaloids such as reserpine, ajmaline and ajma-

licine were determined from *R. serpentina* and *R. vomitoria* by high performance layer chromatography (HPLC) and high performance thin layer chromatography (HPTLC) (Klushnichenko et al., 1994; Srivastava et al., 2006).

In pharmaceutical industries, reserpine is in great demand and mainly extracted from *Rauvolfia* species. Pharmacological studies demonstrate that *Rauvolfia* possesses cardiovascular (Anitha and Kumari 2006), antihypertensive (Von Poser et al., 1990), antiarrhythmic (Kirillova et al., 2001), antiinflammatory (Rao et al., 2012), antipyretic (Amole and Onabanjo, 1999), antidiabetic (Campbell et al., 2006), anticancer (Bemis et al., 2006), hypoglycaemic and hypolipidemic (Qureshi et al., 2009), hepatoprotective (Gupta et al., 2006a), sedative (Weerakoon et al., 1998), antihistaminase (Sachdev et al., 1961), mosquito larvicidal (Das and Chandra, 2012), antibacterial (Ahmed et al., 2002) and antidiarrhoeal (Ezeigbo et al., 2012) activities.

It is also reported that *R. tetraphylla* leaves have potent antibacterial activity against Gram positive and Gram negative bacteria which might be due to the presence of alkaloids (Abubacker and Vasantha, 2011). But no scientific investigation has so far been reported in literature regarding antibacterial activity of *R. serpentina* cultivated in Uttarakhand. Due to high market demand, *R. serpentina* has been introduced for cultivation in the state of Uttarakhand, India, in recent years and successfully grown at farms field with excellent biomass and seeds production capacity.

It is important to analyze the main contents of *R. serpentina* before recommending them for large scale cultivation and medicinal uses. Standardization of herbal drug is also a scientific interest in the herbal drug industry. Considering that, present study was designed to quantify the reserpine content in *R. serpentina* roots and also evaluate antibacterial activity.

## MATERIALS AND METHODS

Roots of *R. serpentina* were collected from Gadarpur farm of Herbal Research and Development Institute, Uttarakhand, India (designated as RS I) and farmer's nursery located at Uttarakhshi (RS II). The plant materials were washed with tap water, cut in small pieces and spread over glass plate to dryness. The dried samples were grinded through pulverizer and particles passed through sieve were taken for extraction and analysis. Standard, reserpine was procured from Sigma Aldrich (Germany), precoated silica gel 60 F<sub>254</sub> TLC plate from Merck and all other chemicals used were HPLC grade.

### Extraction and preparation of samples solution

Hundred milligrams of powdered roots of *R. serpentina* were treated with 1 ml of ammonia for 10 min and then extracted in 10 ml methanol (MeOH). Solvent was removed to dryness under vacuum. The dried extracts were dissolved with 2 ml methanol to make 50 mg/ml solution. Both the extracts were filter through 0.45 µm syringe filter and used for analysis. Accurately 15 µl of each sample was applied to TLC plate.

### Preparation of standard solutions

A stock solution of reserpine (0.1 mg/ml) was prepared in methanol. Different volumes (2, 4, 6 and 8 µl) of the stock solution equivalent to 200, 400, 600 and 800ng were applied to the TLC plates. The calibration curve, correlation coefficient and regression equation were obtained using WinCATS software.

### Instrumentation and chromatographic conditions

The standard and sample solutions were applied on precoated 20 ×10 cm silica gel 60 F<sub>254</sub> plate in the form of bands with 100 µl syringe using automatic sample applicator (Linomat 5). Samples were applied to the plate as 6 mm band, 10 mm apart from Y and 15 mm from X axis using N<sub>2</sub> gas. The slit dimension was 5 × 0.30 mm and scanning speed was 20 mm/s. The plate was developed in a twin trough chamber saturated with mobile phase (chloroform: toluene: ethylacetate: diethylamine). After development, the plate was dried with the help of dryer and observed under UV chamber. The well developed bands of reserpine in standard and *R. serpentina* extracts were scanned at 254 nm in absorption mode with CAMAG TLC scanner controlled by WinCATS software. The source of radiation was deuterium lamp emitting a continuous UV spectrum in the range of 190 to 400 nm.

### Antibacterial activity

Antibacterial activity of *R. serpentina* extracts was determined by well diffusion method according to Deshmukh et al. (2012) with slight modifications. Bacterial cultures of *Salmonella typhimurium*, *Escherichia coli*, *Citrobacter freundii*, *Proteus vulgaris*, *Enterococcus faecalis* and *Staphylococcus aureus* were obtained from Department of Microbiology, HNB Garhwal University, Srinagar, India and used as test organism. All the bacteria were maintained on nutrient agar No. 2 (Himedia, India) at 37°C. The Gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) and Gram negative bacteria (*Salmonella typhimurium*, *Escherichia coli*, *Citrobacter freundii* and *Proteus vulgaris*) were pre cultured in nutrient broth. The stock culture suspensions were diluted with sterile saline water (0.85% NaCl). The Petri dishes were flooded with Mueller Hinton Agar and after solidification of agar 0.1 ml of diluted inoculums were spread over Mueller Hinton Agar (Himedia, India) in the dishes using sterile L spreader to achieve confluent growth of test organism. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Accurately 100 µl of 6.25, 12.5, 25, 50 and 100 mg/ml crude extracts were introduced into the wells, plates were then incubated in refrigerator for about 2 h to allow the diffusion of solution in the medium. After that these plates were incubated at 37°C in incubator for 24 h. Controls were set up in parallel using the solvents that were used to dissolve the extracts. The plates were observed for minimum inhibitory concentration (MIC-lowest concentration of antibacterial that will inhibit the visible growth of microorganism) and zones of inhibition (ZOI). The effects were compared with those of 100 µl Ciprofloxacin at a concentration of 100 µg/ml (10 µg) and the zone of inhibition was measured using antibiotic zone scale.

## RESULTS AND DISCUSSION

High performance thin layer chromatography (HPTLC) was used for the estimation of reserpine (structure in Figure 1) in *R. serpentina*. The standard and sample solutions were spotted in the form of band on the TLC

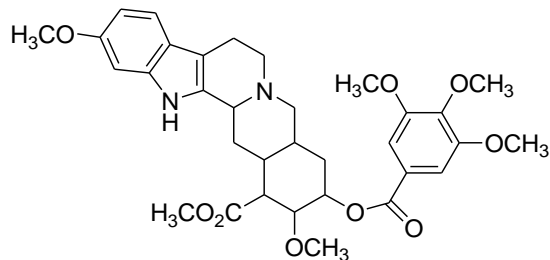


Figure 1. Structure of reserpine.

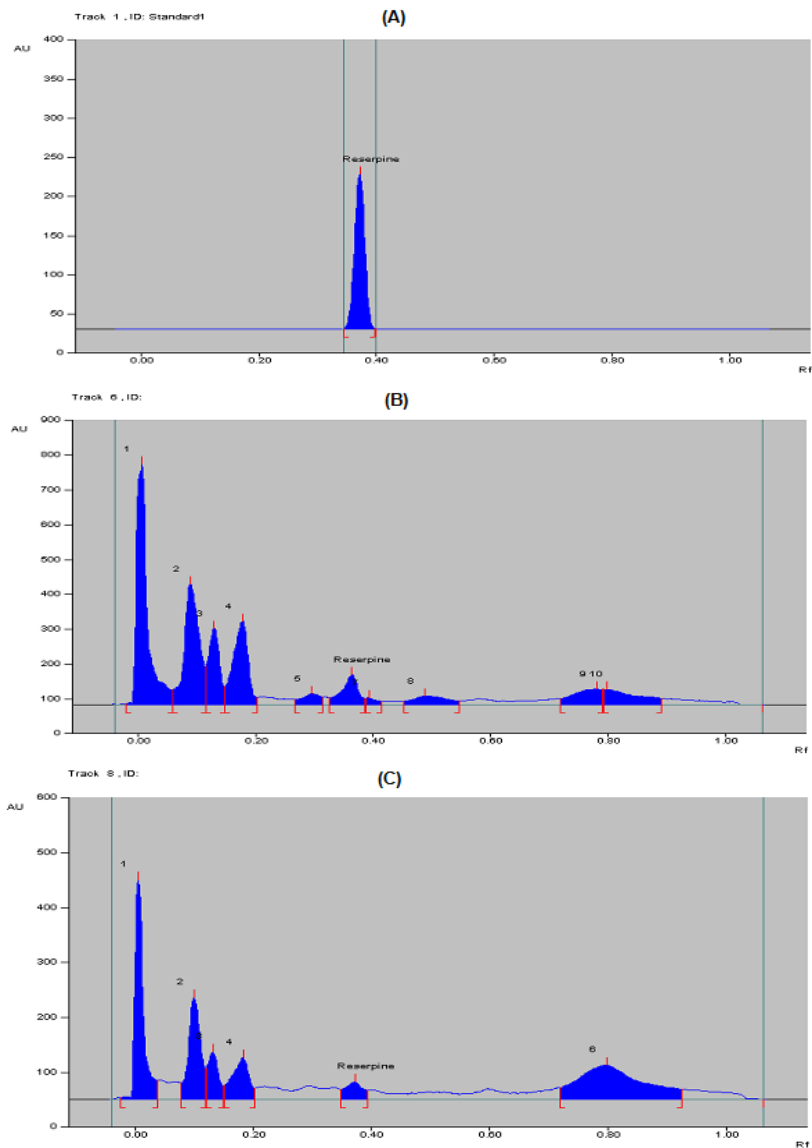


Figure 2. HPTLC Chromatograms of (A) Reserpine, (B, C) *Rauvolfia Serpentina* collected from Gadarpur (RS I) and Uttarakashi (RS II).

plates and run in different solvent systems. The mobile phase consisting of chloroform: toluene: ethylacetate: diethylamine (7:7:4:1) gave well defined bands and sharp peaks. The *r<sub>f</sub>* value and correlation coefficient for reser-

pine was found 0.36 and 0.99, respectively. The chromatogram of standard and samples are shown in Figure 2. The bands of reserpine in samples were confirmed by comparing *r<sub>f</sub>* values with standard. The qualitative results

**Table 1.** Antibacterial activity of *Rauvolfia serpentina* against different test organisms.

Bacterial test organism	Strain no.	RS I		RS II		Standard (Ciprofloxacin)	
		MIC ( $\mu\text{g}/\mu\text{l}$ )	ZOI (mm)	MIC ( $\mu\text{g}/\mu\text{l}$ )	ZOI (mm)	MIC ( $\mu\text{g}/\mu\text{l}$ )	ZOI (mm)
<i>Salmonella typhimurium</i>	MTCC 3224	50	8	50	6	50	-
<i>Escherichia coli</i>	MTCC 443	100	12	100	10	25	18
<i>Citrobacter freundii</i>	MTCC 4221	25	9	25	7	12.5	16
<i>Proteus vulgaris</i>	MTCC 1771	-	-	-	-	25	8
<i>Enterococcus faecalis</i>	MTCC 439	25	9	25	7	6.25	11
<i>Staphylococcus aureus</i>	MTCC 3103	6.25	13	6.25	11	50	10

MIC= minimum inhibitory concentration, ZOI= zone of inhibition.

confirmed the presence of reserpine in both the samples studied. *R. serpentina* collected from Gadarpur was found to contain 0.37% reserpine (dry weight basis) while *R. serpentina* collected from Uttarakashi contain 0.31%. Reserpine has been estimated in *Rauvolfia* species by HPLC and HPTLC. The total reserpine content in *Rauvolfia* species were found 0.06 to 3.0% (Kokate et al., 1998; Gupta et al., 2006b). Kumar et al. (2010) had also quantified the reserpine content of *R. serpentina* collected from different geographical locations of South India. They observed that the reserpine content was ranged from 0.0382 to 0.1442%. Baratto et al. (2012) quantified reserpine content in the dried stem bark of *R. sellowii* by HPLC and found 0.01% dry weight basis. Comparison with previous studies clearly shows that the *R. serpentina* cultivated in Uttarakhand has good reserpine content. This may be attributed to the ambient climatic and topographic conditions of Uttarakhand state.

The methanol extracts of *R. serpentina* exhibited excellent antibacterial activity against tested bacterial organisms as compared to the standard ciprofloxacin. The results were summarized in Table 1. Zone of inhibition are average of triplicate experiments. Sample 1 (RS I) of *R. serpentina* exhibited higher zone of inhibition than Sample 2 (RS II). The highest zone of inhibition (13 mm for RS I and 11 mm for RS II) with lowest MIC (625  $\mu\text{g}$ ) was observed against *Staphylococcus aureus* and highest MIC (10 mg) was observed against *Escherichia coli*, whereas *Proteus vulgaris* was observed resistant upto 10 mg of methanol extract of *R. serpentina*. It was also observed that *R. serpentina* has similar effect towards *Citrobacter freundii* and *Enterococcus faecalis*. Deshmukh et al. (2012) reported antibacterial activity of *R. serpentina* against *S. typhii*, *S. aureus*, *E. coli* and *B. subtilis*.

The methanol extract of *R. serpentina* roots was reported most effective (MIC 40  $\mu\text{g}/\mu\text{l}$ ) against *S. typhii*, moderate against *B. subtilis* (MIC 80  $\mu\text{g}/\mu\text{l}$ ) and least effective against *S. aureus* and *E. coli* (MIC 90  $\mu\text{g}/\mu\text{l}$ ). The petroleum ether extract of *R. serpentina* has been tested for antibacterial activity against Gram positive and negative bacteria and observed 3.0 to 7.8 mm zones of inhibition for Gram positive bacteria and 5.0 to 8.2 mm for

the Gram negative bacteria (Harisaranraj et al., 2009). Comparison of our results with these findings clearly shows that methanol extract of *R. serpentina* is more effective than petroleum ether extract. Antimicrobial activity of *R. tetraphylla* has also been reported, its methanol extract showed 0.25 to 100 mg/ml minimum inhibitory concentration against bacterial pathogens and 0.5 to 100 mg/ml against fungal pathogens (Shariff et al., 2006). Our results demonstrated that methanol extract of *R. serpentina* has concentration dependent antibacterial activity against most of the tested organism.

## Conclusion

*R. serpentina* cultivated in Uttarakhand has good reserpine content and also exhibited moderate to strong antibacterial activity against tested human pathogenic bacteria. Therefore, the species is recommended for large scale cultivation. The results of the study support the folklore claim along with the development of new antimicrobial drugs from the plant. The antibacterial activity of *R. serpentina* may be attributed to the various phytochemical constituents present in the crude extract. Therefore, further work is needed to isolate the active principle from the plant extract which may have even more potency.

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