



## Research article

## Phytochemical screening and antimicrobial activity of leaf, stem, root and their callus extracts in *Rauwolfia Serpentina*

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### ABSTRACT

Phytochemical screening and Antimicrobial studies were carried out in a medicinally important plant species, *Rauwolfia Serpentina*. The aim of the present study was to screen leaf, stem and root extracts and their callus (NaCl stressed & unstressed) extracts of *R. serpentina* (24 months old) for the qualitative analysis of phytochemicals like alkaloids, flavonoids, saponins, tannins and gums and Mucilages and to study antibacterial and antifungal activities in these extracts. Phytochemical screening revealed that Alkaloids are major phytochemicals in leaf, stem and root extracts of *R. serpentina* (24 months old). In antimicrobial activity studies, maximum bacterial growth inhibition zone ( $25.0 \pm 2.4$  mm) was observed in methanolbased leaf extracts against *Staphylococcus aureus* and maximum fungal growth inhibition zone ( $22.0 \pm 2.1$  mm) was observed in *Fusarium Oxysporum* in methanol based root extracts. In NaCl stressed callus extracts both antibacterial and antifungal activities were observed but the results were not comparable to extracts of in vivo plant based leaf.

**Keywords:** Phytochemical screening, Antimicrobial activity, Callus extracts, *Rauwolfia serpentina*.

### INTRODUCTION

Medicinal and aromatic plants (MAPs) have been the focus of study in terms of their conservation and traditional usage in herbal medicines [1]. Throughout the history of mankind, many infectious diseases have been treated with herbs. Medicinal and aromatic plants possess aromatic compounds in the form of oils, which are volatile at the room temperature and the specific property found among them act as a cure for several diseases. These plants have traditionally been used as raw materials for extraction of essential oils, as well as source of spices and other natural products such as traditional herbal medicines, pharmaceuticals, cosmetics, botanical pesticides, insect repellents, other herbal products etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes [2]. It has been estimated that in developed countries such as United States, plant drugs

constitute as much as 25% of the total drugs, while in the developing countries such as China and India, the contribution is as high as 80% [2]. Thus, the economic importance of medicinal plants is much more in India than the rest of the world and the Indian subcontinent constitutes a rich repository of medicinal plants that are used by various indigenous health care systems. As per the estimate, over 7000 species of medicinal plants are used for medicinal purposes [3]. Medicinal plant, *Rauwolfia serpentina* is a small, woody shrub of 60-200 cm height in the Solanaceae family. *Rauwolfia serpentina* is locally known as Ginseng and Ashwagandha. It can be found in the India, Mediterranean, and Africa. The roots of this plant are mainly used therapeutically [4, 5]. Withanolides, which are the active pharmaceutical ingredients, are isolated from the root and leaves of *Rauwolfia serpentina*. Recently, the plant was found

to show antibacterial activities [6, 7, and 8]. Besides antibacterial activities it also exhibited immune modulatory and antitumor activity [9, 10, and 11]. Root extract of *Rauwolfiaserpentina* can reverse Alzheimer's disease pathology via the peripheral clearance of  $\beta$ -amyloid [12]. The water extract from the leaves of *Rauwolfiaserpentina* protect RA differentiated C6 and IMR-32 cells against glutamate-induced excitotoxicity [13]. *Ashwagandha* was found to have anti-carcinogenic effects. Research on animal cell cultures has revealed that the herb reduces the intercellular Tumor, necrosis factor, decreases the levels of the nuclear factor kappa B and potentiates apoptotic signaling in cancerous cell lines [14]. *Ashwagandha* also has capacity to fight cancers by reducing tumor size [15, 16]. *Rauwolfia serpentina* is an important medicinal plant of the world. There is a great demand for its roots, and indiscriminate uprooting from wild sources brought the plant to the verge of extinction. Presently all supplies of *Rauwolfia serpentina* roots are being met from the natural resource, which is declining due to overexploitation by tribal and local collectors. This has led to listing of this species as "endangered" by the International Union for Conservation of Nature and Natural Resources (IUCN) [17]. In India, it has also become an endangered species due to overexploitation and Government of India has prohibited the collection of plants growing in wild in forests and its export since 1969. ~ 1 ~ International Journal of Herbal Medicine <http://www.florajournal.com>. In this scenario, there is urgent need for developing its conservation strategy. *Rauwolfia serpentina* plant commonly known as *Sarpagandha* is widely used medicinally both in the modern medical system and also in Ayurveda, unani and folk medicine. Roots are main source of drug. *Rauwolfia serpentina* is a rich source of indole alkaloids of medicinal value such as reserpine, ajmaline, ajmalicine and serpentine which are used in the treatment of circulatory disorders. It helps to reduce blood pressure by dilating blood-vessels, depresses activity of central nervous system and acts as a hypnotic. In Ayurveda, its roots and whole plants are used for the treatment of cardiovascular disorder, snake bite, rheumatism, hypertension, insanity, epilepsy and leaves are used in removal of opacities of cornea [18, 19]. It is used in traditional medicine in India, Root of *Rauwolfia serpentina* is bitter, acrid, laxative, anthelmintic, thermogenic, diuretic and sedative. The root bark has more than 90% of the total alkaloids in roots. The alkaloids are

accumulated in the roots over a period of 1-3 years and total content varies from 1.0-3.0% of the dried roots. More than 50 alkaloids have been reported from *Rauwolfia serpentina*. The alkaloids are classified into 3 groups, viz, reserpine, ajmaline and serpentine groups. Reserpine group comprises of reserpine, rescinnamine, deserpine etc. Ajmaline, ajmalicine, Ajmalinine, iso-ajmaline etc. are of the ajmaline group. Whereas, serpentine group includes serpentine, serpentininealstonine etc. Quantitative analysis of reserpineRescinnaminegroup of alkaloids is performed by spectrometric analysis or by high-performance liquid chromatography. The roots also contain ophioxilin, resin, starch and wax. So the present study was undertaken to showcase the presence of various phytochemicals in *Rauwolfiaserpentina* and *Rauwolfia serpentina* [20].

## MATERIALS AND METHODS

### Plant Material

The plant materials were collected from Bhopal District of Madhya Pradesh in India during the period of October to November 2024. The shade dried plant powders (100 g) were successively extracted with ethanol by soxhlet apparatus and is used as test sample.

### Phytochemical Screening

The portion of the dry extract was subjected to the Phytochemical screening using the method adopted by Trease, Evans and Harbourne. Phytochemical screening was performed to test for alkaloids, carbohydrates, saponins, glycosidal sugars, proteins, phytosterols, phenols, flavonoids, triterpenoids, tannins Anthraquinone, Phlobatanins, Coumarins, Emodins, fixed oil and Fats.

### Antimicrobial Analysis

The plant material (leaf, stem and roots) of *R. srpentina* were allowed to dry and grinded by using mortar and pestle. Five to ten (5–10) grams of grinded material was dissolved in 50–100 mL of solvent (Petroleum ether, Benzene, Chloroform and Methanol) and kept overnight in an orbital shaker at 100 rpm and 28°C. The extracts were then filtered with Whatman No. 42 filter paper (125 mm) and the resulting extract filtrate was used for further investigation,

### Methods for Phytochemical Analysis

#### Test for alkaloids

A small portion of the alcoholic extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

**Test for Cardiac Glycosides Legal's Test**

Plant extracts were treated with sodium Nitro Pruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

**Test for Carbohydrates Fehling's Test**

About 2mg of plant extracts was shaken with 10 ml of distilled water, filtered and filtrate was concentrated in separate test tube. An equal part of Fehling's Solution A and B (1 ml) were added to each and allowed for boiling for few minutes. Formation of red or brick colour precipitate indicated the presence of the reducing sugar.

**Test for tannins and phenolic compounds**

Following the method of Kapoor et al. (1969), 10 mL of extract was allowed to dry and later treated with 10 mL of heated 0.7% NaCl solution. It was subdivided into three portions. To one of the portion of the test extract 1% NaCl solution was added, gelatine salt was added to another portion; precipitation in the second one indicates the presence of tannins. Addition of FeCl<sub>3</sub> solution to the extract and gives green or blue green coloured precipitate, which is a positive result.

**Test for phytosterols**

One g of the extract was treated with few drops of glacial acetic acid; followed by 3 mL of acetic anhydride, and at last few mL of concentrated sulfuric acid was added. Green colour formation is the positive test (Ling and Jones, 1955).

**Test for terpenoids (Salkowski test)**

5 ml of each extract was mixed in 2 ml of chloroform, and concentrated H<sub>2</sub>SO<sub>4</sub> (3 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

**Test for tannins**

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

**Anthroquinon**

0.5g plant extract boiled with 10% HCl (5 ml) for few min. in water bath then the reaction mixture was filtered and allow for cooling. 2ml 10% ammonia was added and the mixture then heated, rose pink colour formation indicates the presence of Anthroquinon.

**Phlobatanins**

0.5g Extract dissolved in 1 ml distil water and filter, boil with 1ml of 2% HCl solution, red colour precipitate show the presence of phlobatanins.

**Coumarins**

3ml of 10% NaOH was added to 2ml aqueous extract, formation of yellow colour indicate the presence of Coumarin.

**Test for gums and mucilage**

About 5–8 mL of different extracts was taken and added separately in 15–20 mL of 100% alcohol with continuous mixing and then later on filtered. The resultant precipitate was allowed to dry and examined it for swelling properties which indicates presence of gums and Mucilages (Amelia et al., 2011).

**Determination of Antimicrobial Activity Testing of antimicrobial activity**

The test strains were: *Aeromonas liquefaciens* MTCC 2645 (B1), *Enterococcus faecalis* MTCC 439 (B2), *Klebsiella pneumonia* NCIM 2883 (B3), *Micrococcus luteus* NCIM 2871 (B4), *Salmonella typhimurium* NCIM 2501 (B5), *Vibrio cholerae* MTCC 3906 (B6), *Candida albicans* MTCC 1637 (F1), *Cryptococcus* sp. MTCC 7076 (F2), *Microsporiumcanis* MTCC 3270 (F3), *Trichophyton rubrum* MTCC 3272 (F4). The cultures were obtained from MTCC, Chandigarh and NCIM, Pune, India. Microbial strains were tested for antimicrobial sensitivity using the disc diffusion method (Koperuncholan et al, 2010). The antibacterial and antifungal activity of test samples was analyzed against certain microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively. A sterile cotton swab was used to inoculate the bacterial suspension on surface of agar plate. The 15 and 30 µL of sample coated disc were placed in agar plates, separately. For negative control study, the sterile triple distilled water was used. The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus). After incubation, the zone of inhibition was measured with ruler.

**Antibacterial Assay**

Entophytic fungal extracts were screened for their antibacterial activity by disc diffusion plate method. All the test strains of bacteria were sub-cultured and maintained in nutrient agar media. Streptomycin (0.1µg/ml) was used to compare the antibacterial activity in fungal extract through disc diffusion method as described by Devi et al.

**Antifungal Assay**

For the detection of antifungal activity, Potato Dextrose Agar medium (Table 3) (Japanese Pharmacopoeia, 2007) containing plates were inoculated with fungal organisms. Then wells were formed by using sterile borer followed by addition of 100  $\mu$ L of test samples (petroleum ether, chloroform, benzene, methanol and aqueous leaf, stem, root and their callus extracts) including controls (pure solvents) in each well (6 mm diameter). Incubation of plates for 5 days at 25°C. Sterile water was used as a control. At the end of incubation period, inhibition zone was measured surrounding each of the well loaded with specific solvent extract. Three replicates were maintained to calculate average of zone of inhibition.

### Antioxidant Assay

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing ability of plasma or plants (FRAP) assays were used to determine the number of antioxidants in the RSALE and R-AuNPs. At various concentrations (0.0625–1 mg/mL), the RSALE and R-AuNPs were dissolved in methanol. L-ascorbic acid and Trolox were used as a standard for the study, while methanol was employed as a negative control. The experiment was performed on a BiospectrumKinetics spectrophotometer (Eppendorf in triplicates and mean values were recorded).

**Table 1:** Quantitative phytochemical constituent of *Rauwolfia serpentina*

Phytochemical	Acetone root extract	Acetone stem extract	CHCl <sub>3</sub> root extract	CHCl <sub>3</sub> stem extract
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
phenols	+	+	-	-
Carbohydrate	+	+	+	+
Tannins	+	+	-	-
Saponins	-	+	-	-
Glycosides	+	+	+	+
Terpenoids	+	+	+	+
Proteins	+	+	-	-
Anthroquinon	-	+	-	+

**Table 2:** Antibacterial activity of fungal extract against gram negative *E.coli* and Gram positive *S. aureus*

Isolate	Fungal group	<i>E. coli</i>	<i>S. aureus</i>
Rs-R1	Fusarium sp.	+++	++
Rs-S8		-	-
Rs-L1		-	-
Rs-S7		+	+
Rs-R5	Colletotrichum sp.	+++	+++
Rs-S5		-	-
Rs-L4	Xylaria sp.	-	-
Rs-S3		-	-
Rs-R 7B	Phomopsis sp.	-	-
Rs-RL9		-	-
Rs-S4	Clasporium sp.	++	++
Rs-R2A		-	-
Rs-L8	Alternaria sp.	-	-
Rs-S6		-	-
Rs-S1		-	-
Rs-S11		-	-
Rs-L5	Glemastix sp.	-	-
Rs-S2		-	-
Rs-L3A	Aspergillus sp.	-	-
Rs-L6		-	-

**Table 3:** Phytochemical screening of root and stem extract of *Rauwolfia serpentina* in Acetone

Phytochemical	Morinda lucida		
	Leaves	Bark	Roots
Steroids	++	++	+
Alkaloids	+++	++	+
Terpenoids	+	++	+
Tannins	+++	++	+
Saponins	+	+	+
Flavonoids	+++	++	++
Phenol	++++	+++	++
HNC	+	+	++
Glycosides	++	+	+

++++ = Very heavily present, +++ = heavily present, ++ = present, + = trace. + The qualitative analysis showed strongly present, present and trace amount of the different phytochemical in Moringa Lucida plant part (Table 1) the presence of alkaloids, tannins, glycosides, terpenoids. Saponins, phenols, hydrogen cyanide. Flavonoids and steroids were observed in Morinda Lucida

## DISCUSSION

Plant resource of large amount of drug which is having antibiotic properties in the traditional system and also used extensively by the tribal people worldwide and *Rauwolfia serpentina* where chosen for the experiment as they have been known to have immense medicinal value. Extensive phytochemical analysis of the present study indicated that they presence of various secondary metabolites viz. alkaloid, terpenoid, carbohydrate, saponin etc. in methanolic, chloroform and acetic extract of root and stem of *R. somnifera* and *Rauwolfia serpentina*. This study corroborated the fact that both plants have quality phytochemicals which are having immense medicinal importance as already exhibited by various researchers in different parts of the country [20, 4]. The present study also demonstrated that this part of the International Journal of Herbal Medicine country has rich natural resources of medicinal plants which can be to explore further to showcase their full potential.

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