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Hariom Kumar Singh
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, U.P, India

Amit Alexander Charan
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, U.P, India

Aradhana Irene Charan
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, U.P, India

Sudhanshu Matthew Prasad
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, U.P, India

Correspondence
Hariom Kumar Singh
Department of Molecular & Cellular Engineering, Jacob Institute of Biotechnology & Bioengineering, SHUATS, Allahabad, U.P, India

Antifungal and antibacterial activity of methanolic, ethanolic and acetonic leaf extracts of sarpagandha (*Rauwolfia serpentina*)

Hariom Kumar Singh, Amit Alexander Charan, Aradhana Irene Charan and Sudhanshu Matthew Prasad

Abstract
In this study the antibacterial and antifungal activities of selected plants, *Rauwolfia serpentina* (Sarpagandha) were tested against selected fungi (*Aspegillus niger*, *Fusarium oxysporum*, *Penicillium notatum* and *Trichoderma viride*) and selected bacteria (*Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*) cultures respectively. In evaluating antioxidant property and phytochemical analysis, all two plants were screened for antifungal and antibacterial activities. Antifungal and antibacterial activities were evaluated using well diffusion method. Inhibition of fungal growth and bacterial growth were investigated using PDA and NA well diffusion method. The total flavonoid content in crude methanolic, ethanolic and acetonic extracts and minimum inhibitory, minimum fungicidal and minimum bactericidal concentrations were obtained from *Rauwolfia serpentina* leaves.

Keywords: *Rauwolfia serpentina*, antifungal and antibacterial etc.

1. Introduction
In the ancient literatures such as Charak Samhita and Sushrut Samhita which are known as encyclopedia of ayurvedic medicine herbs are found to have medicinal property. Sarpagandha (*Rauwolfia serpentina*) is a medicinal plant par excellence producing useful alkaloids like reserpine (Sahu, 1983) [10]. Various parts of this plant are used to treat human ailments (Ebadi, 2007) [3], in alternative systems of medicine. The root of *Rauwolfia serpentina* (Apocynaceae) has been used in India for hundreds of years for a host of unrelated ailments. Since 1949, after the English publication of a clinical report by the authoron *Rauwolfia serpentina* therapy in fifty cases of essential hypertension, the plant has gained universal acclamation as a useful therapeutic weapon in high blood pressure (Vakil, 2004) [11]. *Aspergillus niger* is a filamentous fungus that commonly occurs in the environment and is generally regarded as non-pathogenic (Blumenthal, 2004) [2]. *Fusarium* is a genus of filamentous fungi that contains many agronomically important plant pathogens, mycotoxin producers, and opportunistic human pathogens (Nelson and Hansen, 1997) [7]. *Penicillium* is a genus of Ascomycetous fungi of major importance in the environment, food and drug production (Pitt, 1979) [9]. *Trichoderma* species are cosmopolitan fungi, frequently present in all types of soil, manure and decaying plant tissues (Kubicek and Harman, 1998) [5]. *Bacillus cereus* is a spore-forming bacterium that occurs naturally in many kinds of foods and can cause illness in humans (Berthold-Pluta *et al*., 2015) [1]. *Escherichia coli* are bacteria that are found in the gut of humans and animals. Most strains of *Escherichia coli* are harmless (Wulf *et al.* 2008) [12]. *Salmonella typhi* causes typhoid fever in humans. Typhoid fever, a systemic febrile illness, is transmitted by the Fecal-oral route, mainly by contaminated food and water in the developing world (Pang *et al.*, 1998)[8]. *Staphylococcus aureus* is a Gram-positive spherical bacterium approximately 1µm in diameter (Heyman, 2004) [4].

2. Materials and Methods
The experiment was conducted at the laboratory of the Department of Molecular and Cellular Engineering, Jacob Institute of Biotechnology and Bioengineering, SHUATS, Allahabad, Uttar Pradesh situated at 25.4131ºN latitude and 18.8479ºE longitude. The fresh leaves of *Rauwolfia serpentina* were collected from the Department of Horticulture and central field, SHUATS, Allahabad which were surface sterilized simply by washing under tap water and Distilled water and dried in shed for 20 days. After drying, leaves and petals of *Rauwolfia serpentina* were grounded in a grinder mixer to powdered form and stored for further use.

The antifungal activity of plant leaves was tested against the selected fungi *viz. Aspegillus niger, Fusarium oxysporum, Penicillium notatum* and *Trichoderma viride* and selected bacteria *viz. Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Salmonella typhi* cultures respectively. Fungal and bacterial cultures were collected from Microbial Culture Collection Bank, SHUATS. The culture was sub cultured on Nutrient agar slants and stored at 4^0C till use. Plant extracts were prepared using organic solvents *viz.* Ethanol, methanol and acetone. Total flavonoid content (Morena *et al*., 2000) [6] was also determined for *Rauwolfia serpentina.*

3. Results and Discussion

Plant extracts were prepared from dried powdered samples. Ethanolic, methanolic and acetonic extracts were taken to study the antifungal and antibacterial activity of the leaves of *Rauwolfia serpentina.* Distilled water was taken as control. Well diffusion method was used in this present study in order to get the antifungal properties of the different plant extracts against the test organism.

1. Results for antifungal activity of *Rauwolfia serpentina* in ethanolic, methanolic and acetonic extracts

(A.) Antifungal activity of Sarpagandha (*Rauwolfia serpentina*) against *Aspergillus niger*

Table 1 and Figure 1 clearly indicated that the ZOI for methanolic extract of *R. serpentina* was lowest against *Aspergillus niger* and ethanolic extract showed maximum ZOI against *Aspergillus niger* whereas acetonic extract showed minimum zone of inhibition against *Aspergillus niger*. DW (control) showed no ZOI.

Table 1: Antifungal activity of *Rauwolfia serpentina* against *Aspergillus niger*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	14 ± 1.0	22 ± 1.0	20±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

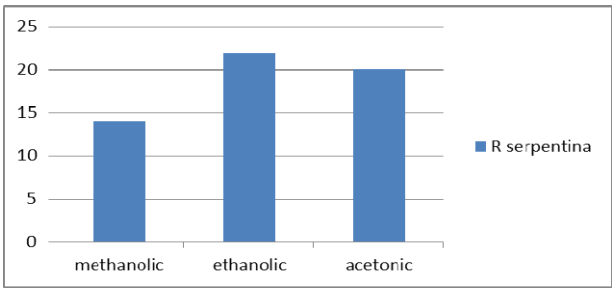

Fig 1: Antifungal activity of *Rauwolfia serpentina* against *Aspergillus niger*

(B.) Antifungal activity of *Rauwolfia serpentina* against *Fusarium oxysporum*

Table 2 and Figure 2 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was lowest against *Fusarium oxysporum* and ethanolic extract showed maximum ZOI against *Fusarium oxysporum* whereas acetonic extract showed minimum zone of inhibition against *Fusarium oxysporum*. DW (control) showed no ZOI.

Table 2: Antifungal activity of *Rauwolfia serpentina* against *Fusarium oxysporum*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	14±1.0	18 ± 1.0	16±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

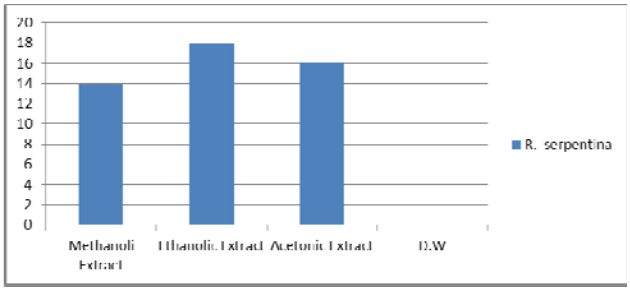

Fig 2: Antifungal activity of *Rauwolfia serpentina* against *Fusarium oxysporum*

(C.) Antifungal activity of *Rauwolfia serpentina* against *Penicillium notatum*

Table 3 and Figure 3 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was lowest against *Penicillium notatum* and ethanolic extract showed maximum ZOI against *Penicillium notatum* whereas acetonic extract showed minimum zone of inhibition against *Penicillium notatum.* DW (control) showed no ZOI.

Table 3: Antifungal activity of *Rauwolfia serpentina* against *Penicillium notatum*

Solvents / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	15 ± 1.0	20 ± 1.0	18±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

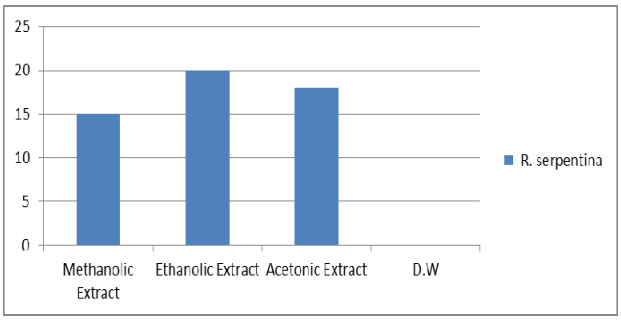

Fig 3: Antifungal activity of *Rauwolfia serpentina* against *Penicillium notatum*

(D.) Antifungal activity of *Rauwolfia serpentina* against *Trichoderma viride*

Table 4 and Figure 4 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was minimum against *Trichoderma viride* and ethanolic extract showed maximum ZOI against *Trichoderma viride* whereas acetonic extract showed comparatively lowest zone of inhibition against *Trichoderma viride*. DW (control) showed no ZOI.

Table 4: Antifungal activity of *Rauwolfia serpentina* against *Trichoderma viride*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	20 ± 1.0	22 ± 1.0	18±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

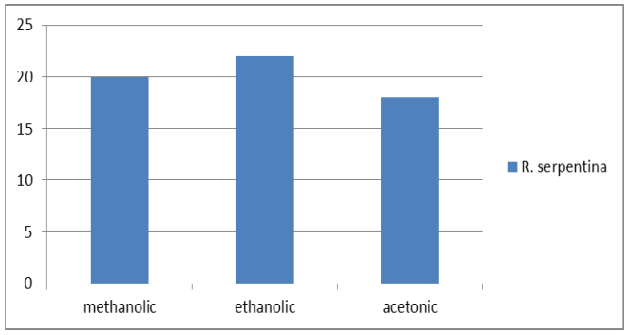

Fig 4: Antifungal activity of *Rauwolfia serpentina* against *Trichoderma viride*

2. Results for antibacterial activity of *Rauwolfia serpentina* in ethanolic, methanolic and acetonic extracts:

(A.) Antibacterial activity of *Rauwolfia serpentina* against *Bacillus cereus*

Table 5 and Figure 5 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was maximum against *Bacillus aureus* and ethanolic extract showed minimum ZOI against *Bacillus aureus* whereas acetonic extract showed maximum zone of inhibition against *Bacillus aureus*. DW (control) showed no ZOI.

Table 5: Antibacterial activity of *Rauwolfia serpentina* against *Bacillus cereus*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	10 ± 1.0	9 ± 1.0	10±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

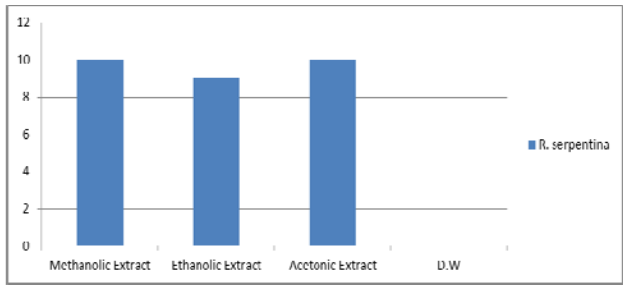

Fig 5: Antifungal activity of *Rauwolfia serpentina* against *Bacillus cereus*

(B.) Antibacterial activity of *Rauwolfia serpentina* against *Escherichia coli*

Table 6 and Figure 6 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was minimum against *Escherichia coli* and ethanolic extract showed maximum ZOI against *Escherichia coli* whereas acetonic extract showed comparatively lowest zone of inhibition against *Escherichia coli*. Distilled water (control) showed no ZOI.

Table 6: Antibacterial activity of *Rauwolfia serpentina* against *Escherichia coli*

Solvent / Plant species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	18 ± 1.0	22 ± 1.0	16±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

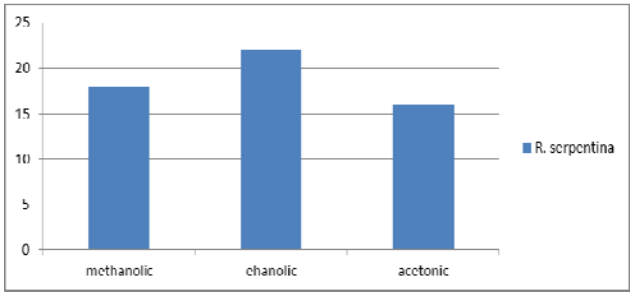

Fig 6: Antibacterial activity of *Rauwolfia serpentina* against *Escherichia coli*

(C.) Antibacterial activity of *Rauwolfia serpentina* against *Salmonella typhi*

Table 7 and Figure 7 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was minimum against *Salmonella typhi* and ethanolic extract showed lowest ZOI against *Salmonella typhi* whereas acetonic extract showed maximum zone of inhibition against *Salmonella typhi*. DW (control) showed no ZOI.

Table 7: Antibacterial activity of *Rauwolfia serpentina* against *Salmonella typhi*

Solvent / Plants species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	18± 1.0	16 ± 1.0	22±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

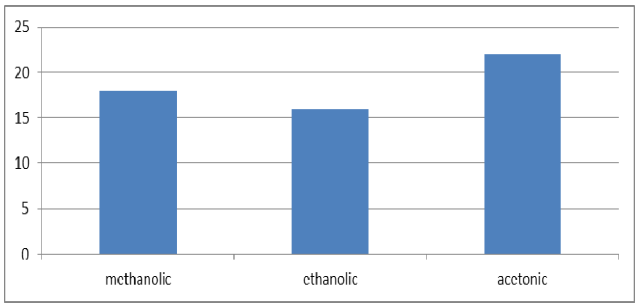

Fig 7: Antibacterial activity of *Rauwolfia serpentina* against *Salmonella typhi*

(D.) Antibacterial activity of *Rauwolfia serpentina* against *Staphylococcus aureus*

Table 8 and Figure 8 clearly indicated that the ZOI for methanolic extract of *Rauwolfia serpentina* was lowest against *Staphylococcus aureus* and ethanolic extract showed minimum ZOI against *Staphylococcus aureus* whereas acetonic extract showed maximum zone of inhibition against *Staphylococcus aureus*. DW (control) showed no ZOI.

Table 8: Antibacterial activity of *Rauwolfia serpentina* against *Staphylococcus aureus*

Solvent / Plant Species	Methanolic extract (ZOI in mm)	Ethanolic extract (ZOI in mm)	Acetonic extract (ZOI in mm)	Distilled water (control) (ZOI in mm)
R. serpentina	10± 1.0	12 ± 1.0	14±1.0	00

(Y axis - ZOI in mm; X axis - Extracts of organic solvent)

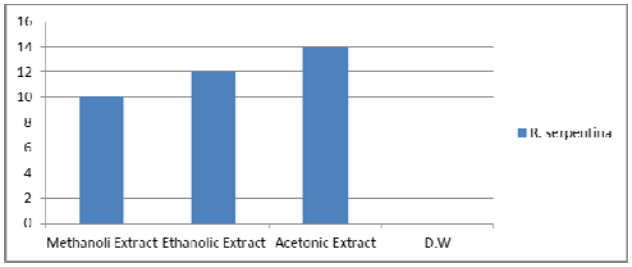

Fig 8: Antibacterial activity of *Rauwolfia serpentina* against *Staphylococcus aureus*

3. Results for total flavonoid content (TFC)

The contents of total flavonoid compounds in crude methanolic, ethanolic and acetonic extracts obtained from curry leaves (*Rauwolfia serpentina*) is presented in **Table 9.** The results were reported as Quercetin Equivalents (QE) mg/g extract.

Table 9: Total flavonoid content in methanolic, ethanolic and acetonic extracts of *Rauwolfia serpentina* sample under study

Plant material	Plant part used	Concentration of plant extract (mg/ml)	O.D. at 415 nm (Methanolic extract)	O.D. at 415 nm (Ethanolic extract)	O.D. at 415 nm (Acetonic extract)	Total flavonoid (mg QE/g extract)
Sarpagandha (*Rauwolfia serpentina*)	Leaves	1.0	1.241	1.506	0.958	0.29

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