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# *In vitro* antifungal activity of ethanol extract of *Rauvolfia tetraphylla* L

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**Abstract**
The present paper deals with in vitro antifungal activity of *Rauvolfia tetraphylla* L. leaves in ethanol extract, was carried out against the test fungi such as *Aspergillus niger, Alternaria alternata, Fusarium oxysporium* and *Macrophomia phaseolina.* Antifungal activity was tested by poison food technique method. These test fungi were found to be sensitive to crude extract as compared to control prepared in ethanol solvent. The result may prove that ethanol extract of leaves found to be possess a good antifungal activity.

**Keywords:** fusarium oxysporum, and macrophamina phaseolina: antifungal activity food poison technique, aspergillus niger, alternaria alternata

## Introduction
A special criteria for angiospermic plants, as they possess large member of phytochemicals as secondary metabolites, having different mechanisms and functions such as chemotherapeutic, bactericidal, bacteriostic, fungicidal and antimicrobial properties ( Purohit and Mathur, 1999) [6].
Among medicinal plants *Rauvolfia tetraphylla* L, commonly called wild snake root or Devil pepper native of tropical America, but cultivated as ornamental plant in garden in India. The plant possess a wide range of alkaloids such as Ajmalicine, Aricine, Renoxidine, Raunescine, Isoraunescine, Deserpidine, Corynanthine, Raujemidine etc. Therefore, the plants may use in Ayurveda Unani and Sidda and pharmaceutical industries as it possess antimicrobial, anti-inflammatory, anti-cancer, anti-psychotic, anti dhirrhoel, anti-hypertensive, anti-oxidant properties. Therefore it is used for high blood pressure patients. Roots are used to stimulate uterine contraction in case of difficulty delivery in Kerala state. Hence, the plant possess large phytochemicals, here an attempt was made to study in vitro anti-fungal activity of *Rauvolfia tetraphylla* L in Ethanolic extract of leaves.

## Methoed and Materials
The fresh leaves of *Rauvolfia tetraphylla* L were harvested from Ch. Shahu Mahavidyalaya, Kolhapur during morning hours for experimental studies.
The harvested leaves were washed in tap water 2-3 times followed by 1-2 times in distilled water; later the leaves were cut into small pieces and sundried for 2-3 days. The dried leaves were powdered in domestic grinder. The dried powered leaves used for experimental study, about 10gm of powdered leaves were soaked in150 ml of ethanol for about 15 days and grinded well with a mortar and pestle. The extract was filtered and subjected to distillation under reduced pressure in rotary evaporator to get a crude extract. This crude extract was subjected to TLC and column chromatography using chloroform and ethyl acetate as solvent in ratio of 95:5. Through column chromatography, four fractions were isolated and used for studying antifungal activity.
Four test fungi such as *Alternaria alternata, Aspergillus niger, Fusarium oxysporium, and Macrophomia phaseolina* procured from Department of Botany, Shivaji University, Kolhapur and maintained in PDA agar media. The antifungal activity was carried out by poison food technique (Ravi kumar Patel *et al.,* 2007) [7] using ethanol different ppm solutions were prepared of the crude extract. The test fungi were allowed to grow on poisoned with 1ml of extract solutions in three different concentrations (100 ppm, 250 ppm, and 500 ppm) on different petriplates. These petriplates were incubated for 48 hrs. and average inhibition zone was calculated in mm and compared with control (solvent – ethanol ).

## Result and Discussion
The result were depicted in Table.1.The test fungs were found to be more sensitive both in crude extract as well as in different fractions at 500 ppm concentration. Meanwhile a very less zone of inhibition was recorded in fractions-1 against *Alternaria alternaria* (5mm) followed by fractions-II, IV and III. This indicates that alcoholic extract was more potent possess fungicide property as compared to crude extract and control. A considerable inhibition zone was found at 100 pm in all fractions against *Fusarium oxysporum* and *Alternaria alterneta.* A parallel result was recorded by Nayeemulla Sharif. *et al*., (2006) [3] in *Rauvolfia tetraphylla* L.
Besides a negligible zone of inhibition was observed in all fractions at 500 pmm concentration against *Fusarium oxysporum f*ollowed by *Aspergilus niger* as compared to control. A similar findings was documented by Nagaraja *et al*., (2009) [5] in *Mappia foetida* against *Aspergilus niger* and *Cladosporium herbarum.* A subtraction zone of inhibition (18.6mm) was documented at 500 ppm concentration in fractions-1 against *Macrophomia phaseolina* and no zone of inhibition in fractions-1 proves that the constituents possess several potent antifungal compounds. Simultaneously a similar zone was found in all fractions against *Fusarium oxysporum* at 250 ppm concentration. A parallel result was

showed by Nagaraja *et al*., (2008) [4] in *Barriingtonia acutangula* against *Fusarium oxysporum* and *Macrophomia phaseolina.*

The extract of higher plants act as a renewable source of antibiotics against bacteria and pathogenic fungi (Fridous *et al*., 1990) [1]. Therefore phytochemicals extracted in different solvents act as potential agents of biopesticide and therapeutics. Concurrently aqueous extract of *Nerium oriander f*ound to be inhibit the germination of *Alternaria solani* reported by Khallil (2001) [2]. Thus the present study may be helpful in preparing different formulations of plant for management of plant disease as Eco-friendly biopesticide.

**Table 1:** Ethanolic extract of leaves of *Rauvolfia tetraphylla* L Against fungi

| Test fungi | Mean diameter of crude extract | Mean diameter of control (ethanol) | Mean of zone of inhibition of different fractions (mm) at different dilutions. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fraction-I | | | Fraction-II | | | Fraction-III | | | Fraction-IV | | |
| *Aspergillus niger* | 27.6 | 41 | 100 ppm | 250 ppm | 500 ppm | 100 ppm | 250 ppm | 500 ppm | 100 ppm | 250 ppm | 500 ppm | 100 ppm | 250 ppm | 500 ppm |
| | | | 40 | 37.6 | 24.3 | 37.6 | 32.6 | 31.3 | 32.6 | 33.3 | 25.3 | 38.01 | 33.02 | 31.30 |
| *Alternaria alternata* | 5.1 | 19.6 | 15.3 | 12.0 | 5.02 | 15.3 | 13.0 | 8.01 | 13.6 | 13.3 | 11.3 | 15.01 | 12.30 | 10.01 |
| *Fusarium oxysporium* | 9.01 | 28.02 | 22.0 | 20.3 | 13.6 | 23.6 | 22.6 | 18.6 | 24.01 | 19.3 | 15.62 | 20.01 | 19.32 | 16.66 |
| *Macrophomia phaseolina* | 10.01 | 70.02 | 70.0 | 20.0 | 0.9 | 47.3 | 40.0 | 38.3 | 50.31 | 41.3 | 34.14 | 33.31 | 30.62 | 18.63 |

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