

Studies on *In Vitro* Antimicrobial Activity of Ethanol Extract of *Rauvolfia tetraphylla*

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ABSTRACT

The antimicrobial activity of ethanol extract obtained from *Rauvolfia tetraphylla* was tested against bacterial species of *Escherichia coli* ATCC 69314, *Streptococcus lactis* NCIM 50038, *Enterobacter aerogenes* NCIM 2340, *Alcaligenes faecalis* ATCC 15246, *Pseudomonas aeruginosa* NCIM 2200, *Proteus vulgaris* ATCC 6380 and fungal species of *Fusarium oxysporum* NCIM 1008, *Alternaria helianthii* ATCC 201540, *Curvularia lunata* ATCC 34477, *Aspergillus niger* NCIM 1207 and *Penicillium spp* NCIM 741. Better antimicrobial activity was observed when the extracts showed maximum activity against *E. coli*, *Enterobacter aerogenes*, *Alcaligenes faecalis*. Among different fungi tested *A. niger* and *Penicillium spp* were found to be more sensitive to crude extract when compared to others.

Key Words: Antibacterial, Antifungal, Ethanol extract, *Rauvolfia tetraphylla*.

Introduction:

Medicinal plants as a group comprise approximately 8000 species and account for around 50% of all the higher flowering plant species of India. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian System of Medicine in the oral and Codified streams use medicinal plants in preventive, promotive and curative applications. There are estimated to be over 7800 manufacturing units in India. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Krishnaraju A V, et al, 2005). Thus it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of the bacterial infections (Balandrin M F, et al 1985).

Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests (Suffredini et al., 2004). The systemic screening of antimicrobial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant pathogenic bacteria and fungi. A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. The so-called secondary metabolites (Evans et al., 1986), which are divided into different categories based

on their mechanism of function like chemotherapeutic, bacteriostatic, bactericidal and antimicrobial (Purohit and Mathur, 1999). The accumulation of phytochemicals in the plant cell cultures had been studied for more than thirty years and the generated knowledge had helped in realization of using cell cultures for production of desired phytochemicals (Castello et al., 2002).

The antimicrobial activity of Solanaceae and Apocynaceae members were well documented in the literature. These include *Cestrum diurnum* (Bhattacharjee et al., 2005), *Capsicum annum* (Cichewicz and Thorpe, 1996), *Withania* spp. (Ramzi et al., 2005), *Picralima nitida* (Nkere and Lroegbu, 2005), *Nerium oleander* (Hussain and Gorski, 2004), *Alstonia macrophylla*, *Alstonia cholaris*, *Voacanga foetida*, *Wrightia* spp. (Hadi and Bremner, 2001), *Rauvolfia serpentina* (Siddique et al., 2004).

The leaf extract of the herbaceous plant, *Rauvolfia tetraphylla* (Apocynaceae), is used for treatment of cholera, eye disease and fever. It is also used as antihypertensive, as well as in intestinal disorders, diarrhea and dysentery (Anonymous, 1969). The principle aim of the present work was to study the antimicrobial activity of *Rauvolfia tetraphylla*, a photochemical study based on the in-vitro screening of ethanol crude extract was done.

Plant material

The fresh matured leaves of the *R. tetraphylla* were collected randomly during the month of January-February, from the Kolli Hills, Namakkal (District), Tamilnadu, India. The plant species were identified by referring the standard morphological characteristic features (keys) according to the Flora of Madras Presidency.

Preparation of Extracts

The dried Leaves were powdered and soaked in the ethanol for about 10-15 days then this cold extract is subjected to distillation at low temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract. Likewise, the powdered leaf which is subjected to soxhlation is exhaustively extracted with ethanol for 48 hours. The solvent was distilled off at lower temperature under reduced pressure in rotary flash evaporator and concentrated on water bath to get the crude extract.

Disc diffusion method

The bioassay for bacterial strains was employed by disc diffusion method (Ergene *et,al* 2006). Filter paper discs (Whatman No. 1) of 5 mm diameter were loaded with crude extracts. Discs were completely dried and sterilized. 100 µl of cultures were spread on sterilized nutrient agar media; impregnated discs were placed on it and incubated for 24 hrs at 37°C. Streptomycin discs (10 µg/disc) were used as a standard drug. The diameter of zone of inhibition in mm was recorded after incubation. The experiment was performed in triplicates and average diameter of zone of inhibition was obtained.

Screening of antifungal activity

The antifungal activity was determined by the poison food technique. The test fungus is allowed to grow on poisoned plate with ethanol extract. It was observed that reduction in colony diameter and extent of sporulation. The effect of sample on the fungal growth was determined by measuring the diameter of the colony obtained on poisoned plate.

RESULTS AND DISCUSSION

The disc diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition around the disc. Among bacterial forms tested, *E. coli*, *Enterobacter aerogenes* and *Alcaligenes faecalis* were found to be more sensitive to crude extract. Other bacterial forms were inhibited by the extract. The zone of inhibition increased on increasing the concentration of extract in disc. This showed the concentration dependent activity (Table 1). The results of poison food technique revealed antifungal nature of the constituents present in the crude leaf extract of *Rauvolfia tetraphylla*. Among different fungi tested *A. niger* and *Penicillium spp* were found to be more sensitive to crude extract when compared to others. A considerable reduction in the sporulation was also recorded.

The antibacterial activity crude extract is shown in Table 1. The extracts showed maximum activity against *E. coli*, *Enterobacter aerogenes* and *Alcaligenes faecalis*. These data revealed that extracts of *R. tetraphylla* exhibited significant antibacterial activity. In testing, inhibition zone increased with increase in drug concentrations and thus exhibiting concentration dependent activity. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavanoids (Tsuchiya *et al.*, 1996), phenolics and polyphenols (Mason and Wasserman, 1987), tannins (Ya *et al.*, 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes (Goren, 1996) etc., are effective antimicrobial substances against a wide range of microorganisms.

Table 1. Antibacterial activity of *Rauvolfia tetraphylla*.

Sl. No.	Organism	Plant extract used	Average Inhibition zone in mm		
1	<i>Escherichia coli</i>	Ethanol extract from seeds of <i>Rauvolfia tetraphylla</i>	26.8	13.3	10.6
2	<i>Streptococcus lactis</i>		24.3	12.6	10.9
3	<i>Enterobacter aerogenes</i>		29.6	14.9	11.5
4	<i>Alcaligenes faecalis</i>		21.2	13.3	10.4
5	<i>Pseudomonas aeruginosa</i>		20.6	11.4	9.6
6	<i>Pseudomonas aeruginosa</i>		25.6	22.5	18.2

Table 2. Antifungal activity of *Rauvolfia tetraphylla*.

Sl.No.	Test organism	Average colony diameter in mm in control plates	Average colony diameter in mm in plates with

			extract
1	<i>Fusarium oxysporum</i>	33	26
2	<i>Alternaria helianthii</i>	36	31
3	<i>Curvularia lunata</i>	39	28
4	<i>Aspergillus niger</i>	24	12
5	<i>Penicillium spp</i>	29	14

CONCLUSION

The antimicrobial activity of *R. tetraphylla* may be attributed to the various phytochemical constituents present in the crude extract. The purified components may have even more potency with respect to inhibition of microbes. The work carried was a basic approach to find out the antimicrobial activity in *R. tetraphylla*. Further works on the types of phytoconstituents and purification of individual groups of bioactive components can reveal the exact potential of the plant to inhibit several pathogenic microbes.

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