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Antimicrobial Activity of *Rauwolfia Serpentina*

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INTRODUCTION

The root of *Rauwolfia serpentina* (Apocyanaceae) is being used as traditional medicine for snake bite, epilepsy and stomach ache by the tribals of Visakhapatnam district, Andhra Pradesh, India (Girijan Cooperative Corporation, Visakhapatnam, Andhra Pradesh, India). It is also manufactured as Ayurveda medicine by many pharmaceutical companies as a remedy for hypertension. An indole alkaloid named ajmalicidine was isolated from *R. serpentina* of Thai origin and its chemical structure was determined (Lounasma and Tolvaneb, 1994). Later two more compounds such as reserpine and rescinnami have been isolated from *R. serpentina* of Indian origin (Farnsworth, 1998) which are used as antihypertensive and tranquilizer respectively. Recently anti-microbial triterpenes from *Ilex integra* showed broad spectral activity against bacteria and *Candida albicans* (Haraguchi, et. al., 1999). But so far, there are no published reports on antimicrobial activity of *R. serpentina* compounds. Therefore, in the present investigation the antimicrobial activity of *R. serpentina* was tested against certain pathogenic microorganisms of four gram negative bacteria viz., *Yersinia enterocolitica (ATCC 9610), Salmonella typhi (NCTC 8393), salmonella typhimurium (ATCC 23564), Escherichia coli* (uro-pathogenic). It was also tested against a gram positive pathogenic bacterium *Staphylococcus aureus (ATCC 9144)* and the fungi, *Candida albicans (ATCC 2091).*

MATERIAL AND METHODS

R. serpentina plant root was collected from a tribal area called Madagada of Araku mandal, Visakhapatnam district, Andhra Pradesh, India. The dried root was pulverised, mixed in sterile distilled water and used as stock. Every time before use, the stock solution was vigorously shaken in order to get homogeneous suspension. All pathogenic microorganisms were obtained from MTCC, Chandigarh, India. To determine the antimicrobial activity of the drug, various concentrations of crude extract(50-1000µg) in 20µl volume were placed on 0.5mm thick sterile filter paper discs of 5mm diameter, on a nutrient agar plate (4mm thick) which was previously inoculated with 0.2 ml of overnight culture ($7.0x10^9$ cells/each strain). An antibiotic disc vancomycin (30µg, Himedia) was also placed in all plates as a positive control (Lennette, et. al., 1980). Similarly *C. albicans* was cultured on YEPD agar medium (yeast extract peptone dextrose). The plates were incubated at 37°C for 18-24h and the zones of inhibition were measured. Later the MICs were determined by tube dilution method, where duplicates of serial dilutions of drug were made from concentrations ranging from 50-1000 µg in 1ml of sterile nutrient broth. In the control test tube 1 ml of nutrient broth was added without any drug. Then $7.0x10^9$ cells of each bacterial strain in 0.05 ml nutrient broth was added into each test tube including the control. The tubes were incubated at 37°C for 18-24h. The lowest concentration of the drug that inhibited the growth(reduction in the turbidity) of bacteria was considered as MIC. Similarly for determination of MIC to *C. albicans,* YEPD (Hi media) medium was used.

Growth Curves: Single colony of each bacterial type was inoculated in 5ml of nutrient broth and incubated at 37°C for 18-24h. The optical density (Nicas and Iglewski, 1986) of this primary culture was measured at 540nm and used to inoculate the secondary broth cultures as described earlier(Elizabeth, K.M, 1996). The secondary cultures were grown at 37°C, in the presence (750µg/ml) as well as in the absence of *R. serpentina.* At every 2h of interval the samples were collected till 10h whose OD at 540nm was measured. The initial OD of control and drug treated samples was noted before incubation. The initial OD of drug treated sample was deducted from the hourly optical densities(to avoid turbidity due to herbal extract), so that what ever increase in the OD may be considered due

to the increase in the growth of microorganisms. *Y. enterocolitica* was cultured in BHI broth, *C. albicans* in YEPD medium and the rest in nutrient broth.

RESULTS

The results given in tables 1and 2 clearly indicate that *R. serpentina* was more effective against *Y. enterocolitica* and *S. aureus* as the minimal inhibitory concentrations were less for these two bacteria than those of others. Zone of inhibition formed at 1000µg concentration of each microorganism showed that *R. serpentina* was most effective against *Y. enterocolitica* (29mm). But vancomycine showed zone diameter of 18 mm against the above mentioned pathogen. However the antimicrobial activity of *R. serpentina* crude extract (1000µg) was very low when compared to that of vancomycine (30µg) against sensitive pathogens (Table 1). If only a sigle component of *R. serpentina* was involved in this activity, it can be considered as a strong antimicrobial agent as per the present study. But if the antimicrobial activity obtained in the present investigation was due to the combined action of several components, it could be inferred that it's activity is insignificant. Presently, work related to the isolation and phytochemical analysis of active components of *R. serpentina* is under progress.

The bacterial growth curve (Table 2) of *Y. enterocolitica* and *S. aureus* in the presence of *R. serpentina* (750ug/ml) showed absorbance near 0 by 2h and 6h respectively, as the OD at 540nm did not increase over the initial optical densities (OD due to drug + OD due to inoculum). Incase of *Y. enterocolitica*, at 2h, instead of increasing, the OD was found less than the initial OD by 0.025 which was the initial OD of all controls which represents the OD of inoculum. This indicates that the cells present in the inoculum were killed by 2h of incubation in the presence of the drug. But in case of other drug

Table 1: The minimal inhibitory concentrations of R. serpentina against 7.0x10⁵cells of certain pathogenic Microorganisms

S.No.	Organism	MICs µg/ml at 1000µg	Zone of inhibition Vancomycine	Zone at 30µg
1.	Y.enterocolitica	250	29mm	18.0 mm
2.	S. aureus	250	27mm	12.0 mm
3.	S. typhimurium	500	24mm	0.0mm (resistant)
4.	E. coli	500	23mm	15.0 mm
5.	S. typhi	500	20mm	0.0mm (resistant)
6.	C. albicans	500	20mm	0.0 mm (resistant)

Table 2: Growth curves in the presence and absence of R. serpentina (750µg/ml)

S. No.	Organism	Concentration of R. serpentina	OD at 540nm				
			2h	4h	6h	8h	10h
1.	Y.enterocolitica	control	0.10	0.25	0.27	0.15	0.18
	,,	750µg/ml	* 0.00	0.00	0.00	0.00	0.00
2.	S. aureus	control	0.09	0.26	0.28	0.18	0.22
	,,	750µg/ml	* * 0.04	0.05	0.03	0.02	0.02
3.	S. typhi	control	0.12	0.25	0.30	0.18	0.25
	,,	750µg/ml	0.03	0.07	0.12	0.09	0.13
4.	S. typhimurium	control	0.18	0.30	0.33	0.28	0.31
	,,	750µg/ml	0.03	0.06	0.09	0.14	0.16
5.	E. coli	control	0.15	0.32	0.40	0.35	0.38
	,,	750µg/ml	0.05	0.06	0.08	0.09	0.20
6.	C. albicans	control	0.25	0.51	0.63	0.56	0.58
	,,	750µg/ml	0.04	0.15	0.35	0.32	0.38

* For all drug treated hourly samples, the initial OD was deducted from the OD of hourly samples to avoid turbidity due to the crude herbal extract. For Y. enterocolitica, it was found that the initial OD was more than the two hour sample. This indicates the destruction of bacterial cells in the inoculum by the drug within two hours and the situation remained the same till 10th hour

**In the case of S. aureus also the optical density did not increase much

treated and control microorganisms there was an increase in OD of hourly samples compared to the respective initial optical densities. For drug treated samples the initial OD was deducted from the OD of hourly samples to avoid density due to drug. These results indicate that *R. serpentina* was bactericidal against *Y. enterocolitica* and S. aureus but microbistatic towards others (as there was reduction in the growth which was not totally inhibited, when compared with controls).

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KEY WORDS Antimicrobial activity. *Rauwolfia serpentina*. Pathogenic microorganisms.

ABSTRACT The disc diffusion method confirmed the antimicrobial activity of Rauwolfia serpentina at concentrations ranging from 50-1000 µg against 7.0×10^5 cells of *S. aureus, Y. enterocolitica, S. typhi, S. typhimurium, E. coli* and *C. albicans*. The minimal inhibitory concentration (MIC) of *R. serpentina* crude extract was 250 µg/ml as determined by tube dilution method against *S. aureus* and *Y. enterocolitica*. It was found that the MIC in case of *E. coli, S. typhi, S. typhimurium* and *C. albicans* was 500 µg/ml indicating that *R. serpentina* was more effective against *S. aureus* and *Y. enterocolitica* than other pathogens tested. But the zone of inhibition formed at 1000 µg concentration suggests that this herbal extract was much more effective against *Y. enterocolitica* (29mm). These results indicate that *R. serpentina* has a potent broad spectral antimicrobial activity.

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