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Bioevaluation of antibacterial potential of Sarpgandha (*Rauwolfia serpentina*)

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Abstract

The in vitro antibacterial activity of *Rauwolfia serpentina* plant extract has been investigated against *Escherichia coli, Staphylococcus aureus, Salmonella typhi* and *Klebsiella pneumoniae* using the disc diffusion method. The alcoholic extract was found effective against *Staphylococcus aureus* only. The antibacterial activity is attributed to the presence of alkaloids, which was confirmed by gas liquid chromatography and positive alkaloid test. The minimum inhibitory concentration (MIC) was determined by paper disc diffusion method. The results were compared with reference antibiotic tetracycline (one unit solution).

Keywords:- *Antibacterial, Rauwolfia serpentina, Plant extract, Disc diffusion method, MIC*

Introduction

The evaluation of plant extract for their antibacterial activity has been known for more than seventy years (Machat and Kankel, 1920). Various medicinal plants have been used for years in daily life to treat diseases allover the world. The medicinal herbs represent a rich source of antibacterial activity. Herbal medicines are still the mainstay of about 70% of world population for health care (Kaushik and Dhiman,1999). Evaluation of plant extract for their antimicrobial activity has been done by several workers. Ansari (1995) studied effect of plant extract against the pathogen of leaf sheath bright of rice. Chakroborty and Brantner (1999) studied antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*. Earlier literature on antibacterial potential of plants has been reviewed by Kaushik and Dhiman (1999). Antibacterial activity of various plant parts of *Aerva persica* has been tested against human pathogenic bacterial strains and pathogenic fungal species (Gehlot and Bohra, 1998).

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Materials and method

The plant material of *Rauwolfia serpentina* was collected from state Ayurvedic college, Gurukul, Haridwar, and bacterial strains *viz. Escherchia coli* (MTCC-739), *Staphylococcus aureus* (MTCC-537), *Salmonella typhi* (MTCC-531) and *Klebsiella pneumoniae* (MTCC-432) were self purchased from IMTECH, Chandigarh. For the preparation of plant extract, plant material were first washed 2-3 times with tap water and then again with sterilized double distilled water. Finally the surface sterilization was done with 90 % ethyl alcohol. 100 grams of plant material were crushed in ware blender resulting in the formation of a paste which was mixed in 250 mlof absolute ethyl alcohol. Alcoholic extract so prepared was allowed to evaporate at room temperature until 80 ml of this was left. This extract was squeezed through double layer muslin cloth and filtered through Whatman's filter paper no-42 and was centrifuged at 5000 r.p.m. for 20 minutes and was then sterilized by passing through 0.2 micron disposable filters for primary screening of antibacterial testing procedure, 100%, 50% and 20% dilution of extract were taken.

For antibacterial screening and minimum inhibitory concentration (MIC), agar and disc diffusion

method was used (Bauer *et al.*, 1966). In this method nutrient agar medium was prepared and autoclave and than cooled up to 42- 45 °C. To each 100 ml nutrient agar medium 1.0 ml of 24 hrs old bacterial cultures was added from nutrient broth and then shaken properly to ensure complete distribution of microorganisms in the medium.

The culture medium which was already inoculated with bacterial suspension, was poured in Petri dishes when it was in solid phase, Whatman's filter paper no-42 discs, which were already dipped in different dilutions of the plant extract were placed on nutrient agar surface. D.W. and absolute alcohol served as negative control and the standard antibiotic tetracycline (one unit solution) as positive control. After inoculation plates were kept at 30° C for 24 hrs in incubator. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone in mm. The alcoholic extract was tested for presence of alkaloid by Dragondraff's test also followed by Stahi (1969) and Gas liquid chromatography (GLC) which was carried under University Science instrumentation centre, Indian institute of Technology (IIT) Roorkee. In GLC HP-5 column and FID detector 250 °C were used for analysis. This column detected alkaloids from extract and solvent was ethyl alcohol.

Results and Discussion

Results of present investigations clearly indicate that the alcoholic extract of *Rauwolfia serpentina* is effective against *S.aureus* only, and was found non effective against *E.coli, S.typhi* and *K. pneumoniae.* The effective zone of inhibition was 24.5 mm against 100 % concentration, 16 mm against 50 % concentration and 8.5 mm against 20 % concentration (Table – 1). The MIC was reported at 14 % extract concentration against *S.aureus.* The results indicated that the undiluted alcoholic extract more effective in comparision to the antibiotic.

Table 1: The antibacterial effect of *Rauwolfia serpentina* plant extract

Test organism	Inhibition zones in mm								
	Antibiotic zone in mm	Extract zone (A)			Control alcohol zone (B) in mm	D.W. zone (C)	Effective zone of inhibition (A-B)		
		100%	50%	20%			100%	50%	20%
Staphylococcus aureus	18	29.5	21	13.5	5	Nil	24.5	16	8.5
Salmonella typhi	19	-	-	-	5	Nil	-	-	-
Escherichia coli	17	-	-	-	5	Nil	-	-	-
Klebsiella pneumoniae	18	-	-	-	5	Nil	-	-	-

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