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Bioevaluation of *Cascabela thevatica* plant extract

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Abstract

The *In vitro* antibacterial activity of *Cascabela thevatica* plant extract has been investigated against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* using the agar disc diffusion method. The alcoholic extract was found effective against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* but does't show any activity against *Klebsiella pneumoniae*. The antibacterial activity is attributed to the presence of alkaloids, which was confirmed by gas liquid chromatography (GLC) and positive alkaloid test. The minimum inhibitory concentration (MIC) was determined by paper disc diffusion method and the results were compared with reference antibiotic tetracycline (one unit solution).

Keywords : *Antibacterial, Cascabela thevatica, plant extract, disc diffusion method, MIC.*

Introduction

The increasing prevalence of multi drug resistant strains with reduced susceptibility to antibiotics raises the specter of untreatable microbial infections and adds urgency to the search for new infection fighting strategies. The use of plant extracts with known antimicrobial properties can be of great significance in therapeutic treatments.The evaluation of plant extract for their antibacterial activity has known for more than seventy years.(Machat and Kankel, 1920).Evaluation of plant extract for their antimicrobial activity has been done by several workers. Chakraborty (1999), studied antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens.* Kaushik and Kishore (1991) studied effect of alcoholic extract of *Pholidota articulata* Lind. against *Bacillus subtilis, Staphylococcus aureus* and *Klebsiella pneumoniae.* Antimicrobial activity of various plant parts of *Aerva persica* has been tested against human pathogenic bacterial strains and pathogenic fungal species (Gehlot and Bohra, 1998). Antifungal activity of *Cassia fistula* leaf extract has been tested against *Candida albicans* (Singh and Karnwal, 2006).

The microorganisms have developed resistance to many antibiotics. This has created immense clinical problems in the treatment of disease; therefore, there is a need to develop an alternative to these drugs for the treatment of disease. The medicinal herbs represent a rich source of antibacterial activity. (Dhar *et. al.*, 1968).

Material and methods

The plant material of *Cascabela thevatica* was collected from B.H.E.L, Hardwar and the bacterial strains viz *Escherichia coli* (MTCC- 739), *Staphylococcus aureus* (MTCC-737), *Salmonella typhi* (MTCC- 531), *Klebsiella pneumoniae*(MTCC-432) were self purchased from IMTECH, Chandi garh.

For the preparation of plant extract, plant material were first washed with 2-3 times with tap water and then again with sterilized distilled water. Finally the surface sterilization was done with 90% absolute ethyl

alcohol. 100gms of plant material were crushed in ware blender resulting in the formation of a paste which was mixed in 250 ml of absolute ethyl alcohol. Alcoholic extract so prepared was allowed to evaporate at room temp. until 80ml of this was left. This extract was squeezed through double layer muslin cloth and filtered through Whatman filter paper no.42 and was centrifuged at 5000 rpm 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% dilutions of extract were taken. In 100% dilution, no distilled water was added, in 50% extract dilution 50% part of distilled water and in 20% dilution 80% part of distilled water was mixed.

For antibacterial screening and minimum inhibitory concentration (MIC), agar disc diffusion method was used (Kirby and Bauer,1966).In this method nutrient agar medium was prepared and autoclaved and then cooled up to 42-45°C. To each 100ml of nutrient agar medium 1.0ml of 24h old bacterial culture of *E.coli*, *S.aureus, S.typhi* and *K.pneunioniae* was added from nutrient broth and than 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 and 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. Distilled water and absolute alcohol served as negative control and the standard antibiotic tetracycline (one unit solution) as positive control. After inoculation plates were kept at 37° for 24 hrs in incubator. The antibacterial activity was evaluated by measuring the diameter of the inhibition zones in mm.

The alcoholic extract was tested for the presence of alkaloid by Dragondroff's test, also followed by Stahl(1969) and gas liquid chromatography (GLC). which was carried under university science instrumentation center, Indian Institute of Technology (IIT), Roorkee. In GLC HP-5 column & FID detector was used for analysis at 250°C temp. This column detected alkaloids from extract and solvent was ethyl alcohol.

Results and discussion

Results of present investigation clearly indicates that the alcoholic extract of *Cascabela thevatica* is effective against *E.coli, S.aureus* and *S.typhi,* and was found non effective against *K.pneumoniae.*The size of effective zone of inhibition of undiluted(100%) plant extract of *Cascabela thevalica* against *E.coli* measured 9.5mm, *S.aureus* 12.7mm and *S.typhi* 11.7mm (Table-1). The MIC of *Cascabela thevatica* extract against *E.coli* was 33.3% concentration, against *S.aureus* measured 11.3% and *S.typhi* measured against 18.3% concentration (Table-2) *S.aureus* was only bacterium, which was the most sensitive to undiluted (100%) alcoholic extract of *C.thevatica*. In reference to antibiotic the zone of inhibition against *E.coli* were 15mm, against *S.aureus* 16 mm and against *S.typhi* 13mm which was nearly comparable to that at plant extract.

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Table -1 : Determination of the antibacterial activity of *C.thevatica* plant extract and reference antibiotic by disc diffusion method.

Test Organism	*Effective Inhibition zone in mm					
	Antibiotic Zone	Extract Zone			Control Alcohol	Distilled water control
		100%	50%	20%		
Escherichia coli	15	9.5	6.7	-	Nil	Nil
Staphylococcus aureus	16	12.7	10.0	6.0	Nil	Nil
Salmonolla typhi	13	11.7	9.7	6.5	Nil	Nil
Klebsiella Pneumoniae	15	-	-	-	Nil	Nil

*Effective zone of inhibition = Total zone of inhibition – Diameter of the disc(5mm)

Table -2 : Minimum Inhibitory Concentration (MIC) of *C.thevatica.*

Extract	**Name of Organisms**		
Alcoholic Plant Extract	*Escherchia coli*	*Staphylococcus aureus*	*Salmonella typhi*
MIC (in %)	33.3%	11.3%	18.3%

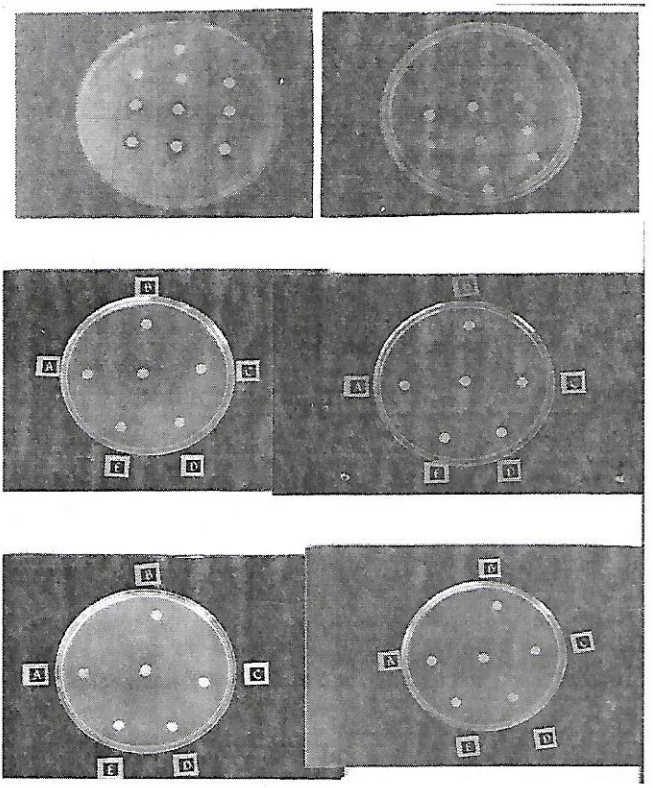

Figure- Showing MIC against *E.coli* and *S.typhi* (Upper)
Showing Zone of Inhibition against *E.coli* (Middle)
Showing Zone of Inhibition against *S.typhi* (Lower)

Here A = Showing 100 % Extract Potency
B = Showing 50 % Extract Potency
C = Showing 20 % Extract Potency
And Center Disc of Antibiotic Potency