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# Antibacterial Potential of *Cascabela Thevatica* Leaf Extract Against *Escherichia Coli*- A Study *In Vitro*

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## Abstract

*Cascabela thevatica* leaf part was tested for antibacterial efficacy against *Escherichia coli* organism using the disc diffusion method. The crude alcoholic extract was found effective against *Escherichia coli* organism. The antibacterial activity is attributed to the presence of alkaloids, which was confirmed by gas chromatography and positive alkaloid test. The minimum inhibitory concentration (MIC) was determined for the crude extract by paper disc diffusion method. The result were compared with reference antibiotic tetracycline (one unit strength).

*Key Words:* ***Cascabela thevatica, Escherichia coli, Apocynaceae, Leaf extract, Antibacterial, Antimicrobial activity.***

## Introduction

It is evergreen and glabrous shrub or small tree with 3-6m height. The leaves are simple, glabrous and narrowed at both ends. It is native of West Indies and Central America, now cultivated in gardens and found along roadsides throughout India. Earlier Literature shows that the seeds are abortifacient and alexeteric and are used as purgative in dropsy and rheumatism. The leaves are emetic and purgative, bark is used in different kinds of fevers (Chopra *et al:* 1956) and the rot in the form of a plaster is applied to tumor (Chaterjee and Pakrashi, 1995). Dinda and Saha, 1990, isolated lupeol, β-amyrin, α-amyrin, γ-teraxasterol and taraxasterol from the root of the plant. The viridoside has also been isolated from stem bark and L-bornesitol from leaves and stem (Chatterjee and Pakrashi 1995).

Evaluation of plant extract for their, anti microbial activity has been done by several workers. Chakraborty and Brantner 1999 studied antibacterial steroid alkaloids from the stem bark of *Holarrhena pubescens*, antibacterial effect of *Rhynchostylis retusa* BL. and *Aerides multiflora* Roxb. (Orchidaceae) were studied by Ghanaksha and Kaushik 1999 a, 1999 b and that of *Adiantum capillus veneris* Linn by Kumar and Kaushik 1999. Ansari 1995 studied effect of plant extract against the pathogen of leaf sheath blight or rice. Kaushik and Kishore 1991 studied the effect of alcoholic extract of shoot extract of *Pholidota articulata* Lindl., against *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Information regarding drug plants and microorganism dated back to Vedic period (Kaushik 1983, 1985, 2000). Gehlot and Bohra 1998 have tested antimicrobial activity of various plant parts of Aerva persica against human pathogenic bacterial strains and pathogenic fungal species. Earlier literature on antibacterial potential of plants has been reviewed by Kaushik and Dhiman 2000. Recently three species of orchids and garlic (*Allum sativum*) studied for the presence of alkaloids and antibacterial potential by Kaushik and Kishore 1991, 95, 97 and Kaushik and Upadhyay 1999, 2000 which showed interesting results.

Investigations into the folkloric antimicrobial activity of *Landolphia owrrience* against *Escherichia coli*, *Bacillus subtilis* and *Candida albicans* was done by Ebi and Ofoefule 1997; chemical composition and anti-microbial activity of *Croton urucurana* Baillon (Euphorbiaceae) against *Staphylococcus aureus* and *Salmonella typhimurium* was done by Peres *et al.* 1997; and anti-microbial properties of *Piper aduncum* against the AIDS related pathogens *Candida albicans*, *Cryptococcus neoformans* and *Mycobacterium intracellular* was done by Okunade 1997. The main aim of the present work is to justify the use of the plant for treatment of infectious diseases.

## Material and Methods

### Plant material

Leaves of the plant *Cascabela thevatica* were collected from the Raja Ji National Park, adjacent to Hardwar.

### Extract preparation

For the preparation of the plant leaf extract, plant leaves 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 gm. leaves of plants were crushed in water blender resulting 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 temperature until 80 ml of it was left. This extract was squeezed through double layer muslin cloth and filtered through Whattman filter paper No. 42 and centrifuged at 5000 r.p.m. for 20 minutes and then sterilized by passing through 0.2 micron disposable filters.

### Selection of test organisms

*In vitro* antibacterial studies were carried out against one Gram negative *Escherichia coli*, MTCC- 739. The strain were self purchased from Microbial Type Culture Collection (MTCC), Chandigarh and further develop in our lab. The bacterial culture was incubated for 24 h at 37 ±2 °C

### Antibacterial testing

The crude ethanolic extract was tested for its antibacterial activity as per the Bauer *et al.* 1966.

### Disc diffusion method

Whattman filter paper No. – 42 discs (ó = 5 mm) were impregnated with 100%, 50% and 20% concentration on the surface of a solid medium (nutrient agar). After solidification of the medium, abstract discs of different concentrations are applied with sterile forceps. After 24 hours incubation, the degree of sensitivity is determined by measuring the zone of inhibition. The experiments were repeated 3 times.

### Minimum Inhibitory Concentration (MIC)

Minimum inhibitory Concentration (MIC) was determined by agar diffusion method. The plant extract was serially diluted with double distilled sterilized water to obtain the desired concentrations and inoculated on agar plate containing pH-6.8 by placing Whattman filter paper No-42 discs (ó = 5mm). After 24 hours the growth was seen. The MIC is defined as the lowest anti microbial concentration of the test compounds, which inhibit bacterial growth. The experiments were repeated 3 times.

### Gas Chromatography (GC)

In phytochemical analysis gas chromatography (GC) was actively carried out. In gas chromatography (GC) HP – 5 column was used. By this column GC detect the alkaloids, fatty acids and drugs etc. and solvent was ethyl alcohol it shows high peak in comparison to all other peaks which was detected by GC in the alcoholic extract ( Figure 1.)

### Alkaloid test

Considering the remarkable results with *C. thevatica* leaf extract, alkaloid test was carried out by leaf extract. For alkaloid presence test, 0.85 gm. bismuth nitrate was dissolved in 50 ml. Acetic acid (20%) and was labeled as solution X. 20 gm. potassium iodide was dissolved in 50 ml. sterilized distilled water and labeled as solution Y and refrigerated in dark up to the time of use because in the presence of air and light the solution gets oxidized. A drop of plant extract was placed in a watch glass and mixed with the equal amount of solution X and Y the presence of alkaloid was confirmed by the appearance of bright orange colour (Stahl 1969). The presence of alkaloids in leaves of *Cascabela thevatica* confirmed antibacterial potential, as alkaloids are said to be antibacterial in nature.

## Results and Discussion

The crude alcoholic leaf extract from *Cascabela thevatica* plant was submitted to an antibacterial screening, using the disc diffusion method. The results are presented as inhibition zone in diameters in Table 1 and as MIC respectively in Table 2. The test sample showed more potent inhibitory effect on Gram bacteria, so the results of present investigations clearly indicate that alcoholic leaf extract of *Cascabela thevatica* possesses antibacterial property. The size of effective zone of inhibition of undiluted extract of *Cascabela thevatica* against *Escherichia coli* measured 14 mm. The MIC of *Cascabela thevatica* extract against *Escherichia coli* was on 35 % concentration. The inhibitory zone measured for antibiotic control is 25 mm for *E. coli*. The results indicated that the antibiotic tetracycline was more effective on *E. coli* in comparison to undiluted alcoholic extract of Cascabela thevatica.

**Table 1. Determination of the antibacterial activity of *C.thevatica* leaf extract, and reference antibiotic by means of the disc diffusion method.**

| Test Organism | Inhibition zone in mm | | | | |
|---|---|---|---|---|---|
| | Antbiotic zone | Undiluted extract zone | Control alcohol zone | Distilled water zone | Effective zone of inhibition |
| | | (A) | (B) | (C) | (A-B) |
| *Escherichia Coli* | 25 | 14 | 5 | Nil | 9 |

**Table 2. Minimum inhibitory concentration ( MIC) of *C. thevatica* leaf extract**

| Pathogen | Escherichia Coli |
|---|---|
| Alcoholic extract concentration (%) | MIC 35 |

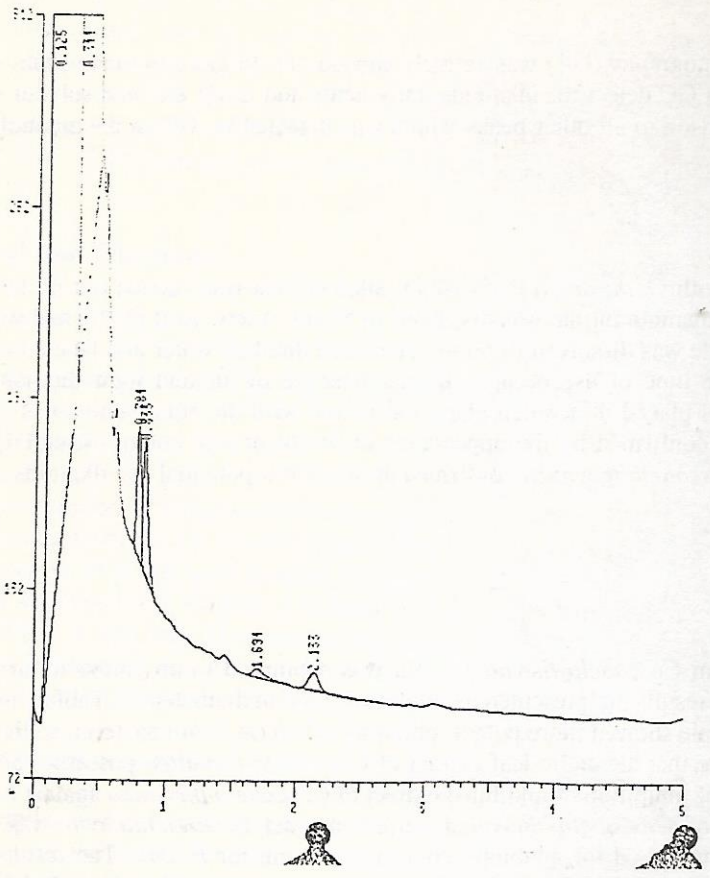


Figure 1. Gas chromatography of *C. thevatica* leaf extract

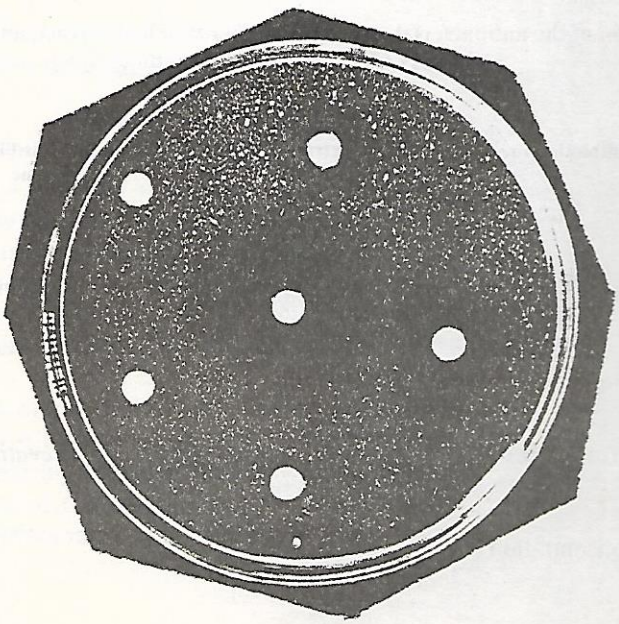

Figure 2. Zone of inhibition against *E. coli*

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