



## Original Research Article

# Antibacterial activity of *Tinospora cordifolia* (Willd) Hook.F.Thoms on urinary tract pathogens

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## ABSTRACT

### Keywords

Antibacterial activity;  
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Antibacterial activity of aqueous, ethanol and chloroform extracts of leaves and stem of *Tinospora cordifolia* Hook.F.Thoms were tested on clinical isolates of urinary pathogens viz., *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by agar well diffusion method. Ethanol extract of leaf showed greater inhibitory action than other tested extracts.

## Introduction

Herbal medicine is still the mainstay of about 80% of the whole population mainly in developing countries for primary healthcare because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developing world (Dharmasiri *et al.*, 2002).

Guduchi [*Tinospora cordifolia* (Willd.) Hook. F. Thoms] is a large, glabrous deciduous climbing shrub belonging to the family Menispermaceae (Nadkarni, 1992). The stem of *Tinospora cordifolia* is rather succulent with long filiform fleshy aerial roots which arise from the branches. The bark is creamy white to grey. The leaves are membranous and cordate. The flowers

are small and yellow or greenish yellow. In auxiliary and terminal racemes or racemose panicles, the male flowers are clustered and female are usually solitary. The drupes are ovoid, glossy, succulent, red and pea sized. The seeds are curved. Fruits are fleshy and single seeded. Flowers grow during the summer and fruits during the winter (Kirtikar and Basu, 1975).

*Tinospora* extracts are widely used in the traditional system of medicine in the treatment of jaundice, rheumatism, urinary diseases, intermittent fevers, eye and liver ailment. It is also an important constituent of many ayurvedic formulations and is reported to possess adaptogenic and immunomodulatory activity in fighting infections (Nayampalli *et al.*, 1982). In the

Indian system it is known to increase the longevity and the bodies' resistance against various diseases (Bhat and Bhat, 1996).

## Materials and Methods

### Collection of plants

The young leaves and stem of *Tinospora cordifolia* were collected from 3-5 months old healthy plant grown in Karaikudi, Tamil Nadu, India, which receives a mean annual rainfall ranging from 100 to 120 cm with an average temperature of 38°C. The pH of the garden soil was 7. The plant leaves and stem were washed thoroughly with tap water followed by sterile distilled water and shade dried at room temperature for 10-15 days.

### Preparation of extracts

The organic constituents from dried plant (Leaf and Stem) material were obtained by continuously extracting the powdered material in soxhlet apparatus with ethanol: water (4:1) as organic solvent for 24 hours at 55°C until complete exhaustion of the material. After completion of extraction, the extracts were passed through Whatman No.1 filter paper and the filtrate was concentrated in vacuum rotary evaporator at 60°C in order to reduce the volume. The paste like extracts were stored in labelled screw capped bottles and kept in refrigerator at 4°C (Natarajan and Francis Xavier, 2003). Likewise extracts were also prepared using the solvent Chloroform: water (4:1) and the aqueous extract was prepared using distilled water and extracted at 100°C.

### Sterility checking

Prior to subjecting the extracts to antibacterial assay they were checked for sterility by inoculating on nutrient agar and incubating at 37°C.

### Specimen cultures

Urine samples were collected from various hospitals in and around Pudukkottai, Tamilnadu, India. Microbes were isolated from the collected urine samples and were subjected to Microscopy and cultural characterization and confirmed as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa* (Cappuccino and Sherman, 1996). The tested bacterial strains were used for further study.

### Standardization of inoculum (WHO drug information, 1993)

The nutrient broth was prepared and well-isolated colonies formed on a cultured agar plate were inoculated into it. It was incubated at 37°C until the culture equals or exceeds the turbidity of a 0.5 McFarland standard. The time usually required was 2 to 8 h.

### Preparation of McFarland solution

This turbidity standard was prepared by adding 0.5 ml of 0.04M BaCl<sub>2</sub> (1.175% w/v BaCl<sub>2</sub> · 2H<sub>2</sub>O) to 99.5 ml of 0.3 N H<sub>2</sub>SO<sub>4</sub> (1% v/v) and was agitated on a vortex mixer just before use. This turbidity standard gave an optical density of 0.08 to 0.10 at 625 nm when tested in a spectrophotometer with 1.cm light path. A McFarland standard with 0.5 turbidity corresponded to an inoculum of 1x10<sup>8</sup> CFU/ml.

### Agar well diffusion method

Antibacterial activity of ethanol extracts of stem and leaf of *T. cordifolia* were carried out by Agar well diffusion method (Perez *et al.*, 1990) using Mueller Hinton Agar medium against the bacterial strains under study. Broth cultures of bacterial strains were swabbed over Mueller Hinton Agar medium using sterile cotton swab and wells were made using sterile well cutter (6mm). Extracts of varying concentrations of 200, 300, and 400 µg/ml solvent were aseptically transferred to the wells separately and incubated at 37°C for 24 hours and the diameter of inhibition zone was recorded. Control wells were maintained with sterile distilled water, ethanol and chloroform.

### Result and Discussion

In the present investigation ethanol extract of leaf of *Tinospora cordifolia* has shown maximum inhibitory activity against *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa* while the Chloroform extract of leaf showed moderate activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* but it has only poor effect against *E.coli* (Table 1). Ethanol extract of stem also showed maximum inhibitory activity against *Klebsiella pneumoniae* while it was moderate against all the other tested pathogen except *Proteus vulgaris*. Chloroform extracts of stem showed maximum zone of inhibition against *Pseudomonas aeruginosa* and moderate inhibition was observed against *Klebsiella pneumoniae* and *E.coli* (Table 2). Aqueous extracts of both leaf and stem showed poor inhibitory activity against all the tested pathogens. *Proteus vulgaris* showed resistance to all the tested extracts.

The clinical conditions caused by urinary pathogens include urinary tract infection, gastro intestinal tract infection, systemic infections, bacteremia and variety of nosocomial infections.

In the present study aqueous, ethanol and acetone extracts of leaves and stem of *Tinospora cordifolia* Hook. F. Thoms were tested on clinical isolates of urinary pathogens under study. The pathogens were sensitive to all the extracts of the plant used (Table. 1).

In recent years there is an increasing incidence of multiple resistances in human pathogenic microorganisms. This is largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in treatment of infectious diseases. The number of resistant strains of microbial pathogen is growing since penicillin resistance and multi resistance in pneumococci caused a major problem in South Africa in 1977. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of uncommon infections are a serious medical problem (Marchese and Shito, 2001). This has forced scientists to search for new antimicrobial substances from various sources like the medicinal plants. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan 2003).

In the present study aqueous and organic extracts showed different results. Among the organic extracts ethanol extracts showed greater activity than chloroform extracts. This is because of most of the

**Table.1** Antimicrobial Activity of leaf extract of *Tinospora cordifolia*

| Name of the microorganism     | Diameter of Zone of inhibition in mm     |     |     |   |     |     |  |     |      |
|-------------------------------|--|-----|-----|---|-----|-----|--|-----|------|
|                               | Aqueous Extract<br>(Concentration in µg) |     |     | Chloroform Extract<br>(Concentration in µg) |     |     | Ethanol Extract<br>(Concentration in µg) |     |      |
|                               | 200                                      | 300 | 400 | 200   | 300 | 400 | 200                                      | 300 | 400  |
| <i>Escherichia coli</i>       | -  | -   | -   | -   | --  | 0.5 | 0.3                                      | 0.5 | 1.0  |
| <i>Klebsiella pneumonia</i>   | -  | 2.0 | 3.0 | 2.0   | 3.0 | 5.0 | 6.0                                      | 9.0 | 12.0 |
| <i>Proteus vulgaris</i>       | -  | -   | -   | -   | -   | -   | -  | -   | -    |
| <i>Pseudomonas aeruginosa</i> | 0.1                                      | 0.3 | 0.5 | 3.0   | 5.0 | 5.5 | 5.0                                      | 6.0 | 9.0  |

**Table.2** Antimicrobial Activity of Stem extract of *Tinospora cordifolia*

| Name of the microorganism     | Diameter of Zone of inhibition in mm     |     |     |   |     |     |  |      |      |
|-------------------------------|--|-----|-----|---|-----|-----|--|------|------|
|                               | Aqueous Extract<br>(Concentration in µg) |     |     | Chloroform Extract<br>(Concentration in µg) |     |     | Ethanol Extract<br>(Concentration in µg) |      |      |
|                               | 200                                      | 300 | 400 | 200   | 300 | 400 | 200                                      | 300  | 400  |
| <i>Escherichia coli</i>       | -  | -   | -   | 0.2   | 0.3 | 0.4 | 0.3                                      | 0.6  | 1.0  |
| <i>Klebsiella pneumoniae</i>  | -  | 2.0 | 4.0 | 3.0   | 5.0 | 8.0 | 6.0                                      | 11.0 | 15.0 |
| <i>Proteus vulgaris</i>       | -  | -   | -   | -   | -   | -   | -  | -    | -    |
| <i>Pseudomonas aeruginosa</i> | -  | 2.0 | 5.0 | 3.0   | 5.0 | 6.0 | 6.0                                      | 9.0  | 12.0 |

antibacterial active principles can be extracted through the solvent ethanol. Similar findings were drawn by Krishna *et al.*, (1997). Comparing the stem and leaf extracts of *Tinospora cordifolia*, stem extracts were effective against tested bacterial strains.

A number of different active principles including alkaloid (berberine), bitter compounds tinosporin, tinosporic acid, tinosporal, essential oil and a mixture of fatty acids have been identified as contributing to the observed medicinal effects in *Tinospora cordifolia* (Wealth of India, 1989).

Further studies need to be undertaken regarding toxicity, safety and absorption pattern of the active ingredients of this plant.

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