

ANTIBACTERIAL POTENTIAL OF *TINOSPORA CORDIFOLIA* AGAINST RESISTANT HUMAN PATHOGENIC BACTERIA

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ABSTRACT

The antibacterial activity of various fruit and stem extracts of *Tinospora cordifolia* was tested against four human pathogenic bacteria namely *Bacillus cereus*, *Bacillus fusiformis*, *Escherichia coli* and *Klebsiella pneumonia* by using paper disc agar diffusion method. The methanolic and aqueous fruit extracts showed significant response to all the pathogens, while aqueous stem extract showed poor activity against the tested bacterial pathogens. Thus, the present study suggests that the aqueous and methanol fruit extracts were more active in comparison to stem aqueous extracts and can be used for the treatment of various human diseases caused by the tested bacteria.

Keywords: *T.cordifolia*, Bacterial pathogens and Antibacterial activity

INTRODUCTION

The medicinal plants have been in the focus as life saving drugs right from beginning of human civilization. Our Vedas the Ramayana and several ancient writings clearly depict the successful use of herbal drugs for human beings. The medicinal and herbal plants have been the object of research in both classical and advanced areas of plant sciences. India has rich diversity of medicinal plants. The supply base of 90% herbal raw drugs used in the manufacture of Ayurveda, Uiddha, Unani and Homoeopathy system of medicine is largely from the wild, this wild source is speedily shrinking day-by-day. Although India has rich biodiversity and is one of the 12 mega diversity centers, the growing demand for medicinal plants is putting a heavy strain on the existing resources, causing a number of species to be either threatened or endangered. IUCN in the year 2000 revealed that India ranked fifth highest in the number of threatened plant species of medicinal plant species and birds globally⁵.

Today, we are witnessing a great deal of public interest in the use of herbal remedies. Furthermore; many drugs had their origin in plant extract. There are many herbs, which are predominantly used to treat cardiovascular problems, liver disorders, central nervous system, digestive and metabolic disorders¹.

Tinospora cordifolia (Menispermaceae) commonly known as Guduchi in India. It is widely distributed in tropical parts of the country. It is a large, glabrous, deciduous, climbing shrub. It has been used for the treatment of various human diseases such as diabetes, jaundice, stomachache, improve the immune system and various types of fever. It helps to remove urinary stones and reduce blood urea.

The purpose of the present study to evaluated the antibacterial activity of fruit and stem extracts of *Tinospora cordifolia* against some resistant human pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant material

The plants of *T.cordifolia* were growing at different sites of Agra region. The stem and fruits were collected, washed thoroughly with running tap water and once with sterile distilled water subsequently. The plant materials were dried on whattman filter paper for two to three weeks in shade. Further, the plant specimens were preserved in the herbarium of R.B.S. College, Agra, U.P.

Preparation of aqueous extracts

The plant samples (stems and fruits) were ground into uniform powder using a grinder. 15gm. of dried powder was taken in 250 ml distilled water in separate conical flasks, air tight with cork and then kept on a shaker for 8 hours. After it the extract were filtered by

using a vacuum filtration system and stored at 4°C in airtight containers.

Preparation of solvent extracts

The dried powder of stem and fruit was passed through whatman filter No. 40 to achieve uniform particle size and then used for extraction process. A weighed quantity of the powder was subjected to continuous hot extraction in soxhlet apparatus with different solvent viz., methanol. The extract was dried using rotator vacuum evaporator and they give molten extract and store at 4°C until further use.

Test Microorganisms and Bacterial Culture

Present investigation was carried out with three strains of human pathogenic bacteria. The bacterial strains include *Bacillus cereus*, *Bacillus fusiformis*, *Escherichia coli* and *Klebsiella pneumonia*. All microorganisms were clinical isolates, obtained from the Microbiology Laboratory, District Hospital Agra and very carefully identified using standard microbiological methods.

(b) Preparation of Inoculums

The dilution assay (broth) was carried out according to⁶. A loopful of the bacterial culture from the slant was inoculated in the nutrient broth (BHI broth as well as MH broth) and inoculated at 37°C for 24 hours. The fresh broth (20ml) was seeded with 0.25 ml of the 24 hour both cultures and a twofold serial dilution method was followed as below. The dried plant extracts were dissolved in 85 % methanol to obtain an 80 mg/ml solution and sterilized by filtration through a 0.45 µm membrane filter. A 0.2 ml solution of the material was added to 108 ml of the seeded broth and this formed the first dilution. 1 ml of this dilution was diluted further with 1 ml of the seeded to produce the second dilution and the process was repeated until six dilutions were obtained. A set of tubes containing only seeded broth was kept as control and 85 % methanol controls were also maintained. After incubation for 24 h at 34°C the last tube with no visible growth of the bacteria was taken to represent the minimum inhibitory concentration (MIC) of the test sample which is expressed in mg/ml. Moreover, the broth dilution assay was carried out with **ampicillin** and gentamicin in BHI broth as well as MH broth in the same way as the extracts and the MIC values of **ampicillin** and **gentamicin** were determined⁴.

(c) Determination of antibacterial activity

Screening of antibacterial activity was carried out by paper disc method³. High media sterile disc were used for activity, saturated disc with the extract (0.04ml) and known quantity of standard reference antibiotic separately were air dried at room temperature. The molten Muller Hinton (hi media) was inoculated with the 100 ml of the inoculums and poured into sterile Petri plates (borosil)

The disc with test compound placed on the upper surface of sterilized Muller Hinton plate that had been inoculated with the test organism (using a sterile swab) and air dried to remove the surface moisture. The thickness of MH medium was kept equal in all Petri plates and the standard disc (Streptomycin) was used in each plate as control². The plates were inoculated 24 hours at 37°C in BOD incubator. After 24 hours growth of bacteria was measured for its zone of inhibition. The results were obtained by measuring the zone diameter. The experiment was conducted in replicates of 3 and the mean value is presented. The results were compared with the control tetracycline and streptomycin.

RESULT AND DISCUSSION

The results obtained on antibacterial activity of *Tinospora cordifolia* by disc diffusion method were summarized in Table-1. It is evident from Table-1 that all extracts of stem and fruit exhibited significant antibacterial activity against the tested bacteria. However, the strongest antibacterial activity was observed in methanol fruit extract of *Tinospora cordifolia* against *Klebsiella pneumoniae* with maximum inhibition zone (22mm). The aqueous fruit extract showed minimum antibacterial activity against *Bacillus cereus* with (10mm) zone of inhibition. The stem extract in the methanol solvent exhibits highest zone of inhibitory growth against *K.pneumoniae* with (18mm) zone of inhibition while least antibacterial activity was recorded against *B.fusiformis* with 9.5 mm zone of inhibition.

From the present investigation on antibacterial activity of *T.cordifolia*, it may be concluded that methanolic fruit extract shows more promising effect on the growth of all tested bacteria, The activity could be attributed to the presence of some medicinally active chemical compounds in fruits further more there is need to the isolation and characterization of phyto compounds for future perspective.

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Table 1: Antibacterial activity of fruit and stem fruit extracts of *T.cordifolia* against human pathogenic bacteria.

S. No.	Bacteria	Zone of Inhibition (mm)				Antibiotic
		MFE	MSE	ASE	AFE	
1.	<i>B.fusiformis</i>	11	9	8	9	10
2.	<i>Bacillus cereus</i>	8	9.5	9	10	12
3.	<i>E.coli</i>	10	12	7	8	10
4.	<i>K.pneumoniae</i>	22	18	10	9	15

ASE= Aqueous stem extract, AFE=Aqueous fruit extract

AFE=Aqueous fruit extract, Antibiotic= Streptomycin

MFE= Methanol fruit extract, MSE= Methanol stem extract

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