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# PHYTOCHEMICAL SCREENING, CHROMATOGRAPHIC STUDIES AND ANTIBACTERIAL ACTIVITY OF *Tinospora cordifolia* PLANT EXTRACTS

Krishnadutt Pratihast*, Rajneesh Kumar**, and Vyomesh Vibhaw*

Key words : *Tinospora cordifolia*, antibacterial activity, phytochemicals, ethanol extracts and zone of inhibition.

The antibacterial activity of *Tinospora cordifolia* extracts on pathogenic bacteria was observed in this study. *Tinospora cordifolia* were extracted using ethanol, chloroform and n-hexane as solvents. The *Tinospora cordifolia* extracts were tested against Bacillus cereus, Bacillus subtillis, Salmonella typhi and Escherichia coli by agar well diffusion method. The extract demonstrated higher activities against tested bacteria with the highest activity (17.23±0.25 mm zone of inhibition). The ethanol fraction was fractionated and yielded 7 fractions. The fraction 5 exhibited highest activity against *E. coli*. Preliminary phytochemical analysis showed that the extracts contain alkaloids, tannins, saponins and phenols. The results justified the use of *Tinospora cordifolia* extracts in the treatment of gastroenteritis, uretritis, typhoid fever and wound infections

## INTRODUCTION

The use of plants for medicinal purposes is as old as human civilization itself. Medicinal plants have been used for curing diseases in different traditional systems of medicine such as Ayurveda, Siddha, European, Tibetan, and Unani. (Sofowora, 1982) Herbal medicine is still the mainstay of treatment in about 75%-80% of people in many developing countries for their primary health care because of better cultural acceptability and compatibility with the human body and fewer side effects. (Blumenthal 1998). One such immensely valuable plant regarding its constituents and pharmacology is *Tinospora cordifolia* of the Menispermaceae family, commonly called as Guduchi in Sanskrit. (Still *et.al.,* 1978) It is a deciduous climbing shrub with small greenish flowers, having enormous medicinal value in all its parts such as leaves, stem, and also the root. (Jonathan 2007). It is a Rasayana (rejuvenator) and anti-aging medicine in Ayurveda, used to improve the immune system and the body resistance against infections. (Onibon et. al., 2007). It has also been found that *Tinospora* has antispasmodic, (Imaga *et al*., 2009) antipyretic, (Nirosha, and Mangalanayaki, 2013), anti-inflammatory,anticomplementary, (Harborne 1998) and immunomodulatory activities. In addition to it, *Tinospora* has been found to exhibit antidiabetic, (Sofowora 1993), hepatoprotective, (Trease and Evans 1989) anticancer, and antioxidant properties as well. It has been listed as an insecticide, an antifungal agent, and an antibacterial agent. Antimicrobial features have been found in its root, stem, and leaf extracts on pathogenic microorganisms. Ethanolic extracts of *T.cordifolia* have been successfully tested against various bacteria such as *Staphylococcus*, *Escherichia coli*, *Aspergillus niger*, *Candida albicans*, and *Staphylococcus aureus*. (Akiyama 2001) . Hence, the present *in vitro* study was undertaken to assess the antimicrobial properties of *Tinospora cordifolia* against different bacterial strains.

## MATERIALS AND METHODS

### Collection and processing of Plant Sample

The plant *Tinospora cordifolia* (commonly known as heart-leaved moonseed, gudduchi, giloy, amrita, guduchika) was collected from different regions in northern India in 2019 and identified according to the relevant monographs of Indian Pharmacopoeia (2012). The plant materials were botanically authenticated at Botany Department of Patna University; Patna. Voucher specimens of each of the plant's extract have been deposited in the department

### Plant Extraction for Phytochemical Assays

100g of the plant sample was dissolved in 400ml of ethanol, n-hexane and chloroform respectively, and left for 48hrs. The extracts were then decanted and filtered through a Whatman filter paper. The filtered extract was then sterilized using a membrane filter and evaporated to dryness at 45°C. The residues obtained were then stored in the refrigerator at 4°C until used.

***Department of Biochemistry, Patna University, Patna-800023, Bihar, India**
****Department of Chemistry, Patna University, Patna-800023, Bihar, India**

**Phytochemical Screening**

The extracts were screened for the presence of major phytochemicals using standard qualitative methods as described previously((Harborne 1998). The plant extracts were screened for the presence of glycosides, phlobatannins, terpenoids, steroids, saponins, tannins, alkaloids, flavanoids, anthraquinones and phenols.

**Collection and maintenance of Test Organisms**

In the present study, the bacteria selected are described in Table 1. Microbial pure cultures were obtained from MTCC (Microbial type culture collection), Chandigarh. The bacterial cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37°C and potato dextrose agar medium (Hi Media, pH 5.6) at 27°C respectively. Both the cultures were maintained at 4°C.

**Table 1:Selected Gram positive and Gram negative microorganisms**

| Types of microorganism | Micro-organism strains | Causes |
|---|---|---|
| **Gram positive** | *Bacillus subtilis* (MTCC 6038) | Food poisoning |
| | *Bacillus cereus* (MTCC 1765) | Food poisoning, vomiting, diarrhoea |
| **Gram negative** | *Escherichia coli* (MTCC 5946) | Bloody diarrhoea, kidney diseases |
| | *Salmonella typhi* (MTCC 8345) | Typhoid, enteric fever |

**Column Chromatography**

Column chromatography was used to get the fraction of plant extracts (Trease and Evans, 1989). (Two gram) of the extract was subjected to column chromatography to separate the extracts into its component fractions. Silica gel (60-120 mesh) was used as the stationary phase, and the solvent system chloroform: ethanol as mobile phase. In the setting up of the column chromatography, the lower part of the glass column was stocked with glass wool with the aid of glass rod. The sample was prepared by adsorbing 2.0g of the extract to 10g of silica gel (60-120 mesh) in ethanol then allowed to dry, the dry powder was gently layered on top of the column then a glass wool was put on top so as to avoid splashing of the solvent system when pouring it into the column. The elution of the extract was done with solvent system chloroform: ethanol 9:1. The eluted fractions were collected in bottles.

**Antibacterial Activity of Column Fractions of *Tinospora cordifolia* Extract.**

This was carried out using agar disc diffusion technique as described by Akiyama 2001). The Muller Hinton agar medium was allowed to solidify, after which the test organisms were aseptically inoculated on different Petri dishes using sterile swab sticks, with the aid of a syringe the fractions were added to the disc of the inoculated plate ethanolic *Tinospora cordifolia* extract. Ciprofloxacin was used as the positive control and distilled water was used as a negative control and was incorporated in the inoculated plates. The discs were sufficiently spaced out to prevent overlapping of the zones. The phytochemical screening, chromatographic studies and antibacterial activity of *Tinospora cordifolia* extracts plates were then allowed for the pre-diffusion time of 15 minutes after which they were incubated for 24 hours at 37° C. Diameters of zones of inhibitions were measured using millimeter rule and the results expressed in millimeter.

**RESULTS AND DISCUSSION**

All the three extracts were subjected to phytochemical analysis, and the results were tabulated (Table-1). Alkaloids, flavonoids, saponins, phenols and tannin were present in ethanol, choloroform and hexane extracts (Table-2).

**Table: 2. Qualitative determinations of active ingredients in ethanolic, hexane and choloroform extracts of Tinospora cordifolia plant**

| Plant Constituents | Extract of Tinospora cordifolia | | |
|---|---|---|---|
| | Ethanolic | Hexane | Chloroform |
| Alkaloids | + | + | + |
| Saponins | + | + | + |
| Flavonoids | + | + | + |
| Fixed oil and fats | + | + | + |

The phenolic content of the ethanolic extract of *Tinospora cordifolia* was found to be 16.33±0.12 mg GAL/g while the phenolic content of the hexane extract was found to be 10.43 ± 0.04 mg GAL/g and 11.43 ± 0.16 mg GAL/g per gram dry weight basis for chloroform extract (Table-3).

**Table:3 Total phenolic content (expressed as mg Gallic acid (GAL) equivalent/g dry weight) of Tinospora cordifolia plant extracts**

| Solvents | Total phenol concentration of Tinospora cordifolia (Mean± S.D) |
|---|---|
| Ethanol | 16.33 ± 0.12 |
| Hexane | 10.43 ± 0.04 |
| Chloroform | 11.43 ± 0.16 |

The flavonoid content of the ethanolic extract of *Tinospora cordifolia* in terms of quercetin equivalent was found to be 2.01 ± 0.08 mg/g(Table-4) while, the flavonoid content of the hexane extract in terms of quercetin equivalent was found to be 1.04 ± 0.08 and for chloroform extract it was found to be 1.86 ± 0.04 mg/g

**Table:4: Total flavonoid content (expressed as mg Quercetin solution equivalent/g dry weight) of Tinospora cordifolia plant extracts**

| Solvent | Total flavonoid concentration of Tinospora cordifolia (Mean± S.D) |
|---|---|
| Ethanol | 2.01 ± 0.08 |
| Hexane | 1.04 ± 0.08 |
| Chloroform | 1.86 ± 0.04 |

The plant extracts showed different degrees of antimicrobial activity depending on the concentration of extracts, type of solvent used for extraction and the bacterial strains tested for susceptibility assay ( Figure 1.1 and Figure 1.3). The collective analysis of antimicrobial activity of extract indicated that medicinal plants used in the study the ethanolic extracts of the plants exhibited better antibacterial activities than other extracts. The ethanolic extract of *Tinospora cordifolia* showed maximum antibacterial activity with maximum diameter of zone of inhibition against the four strains. At 500 mg/ml concentration, the ethanolic extract of *Tinospora cordiofolia* on *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli a*nd *Salmonella typhi* showed maximum zone of inhibition of 11.8±0.26 mm, 16.76±0.25 mm, 17.23±0.25 mm and 16.66±0.15 mm respectively (Figure 1.1)

**Fig: 1.1: Antimicrobial activities of the ethanolic extract of *Tionospora cordifolia* plant in agar diffusion test**

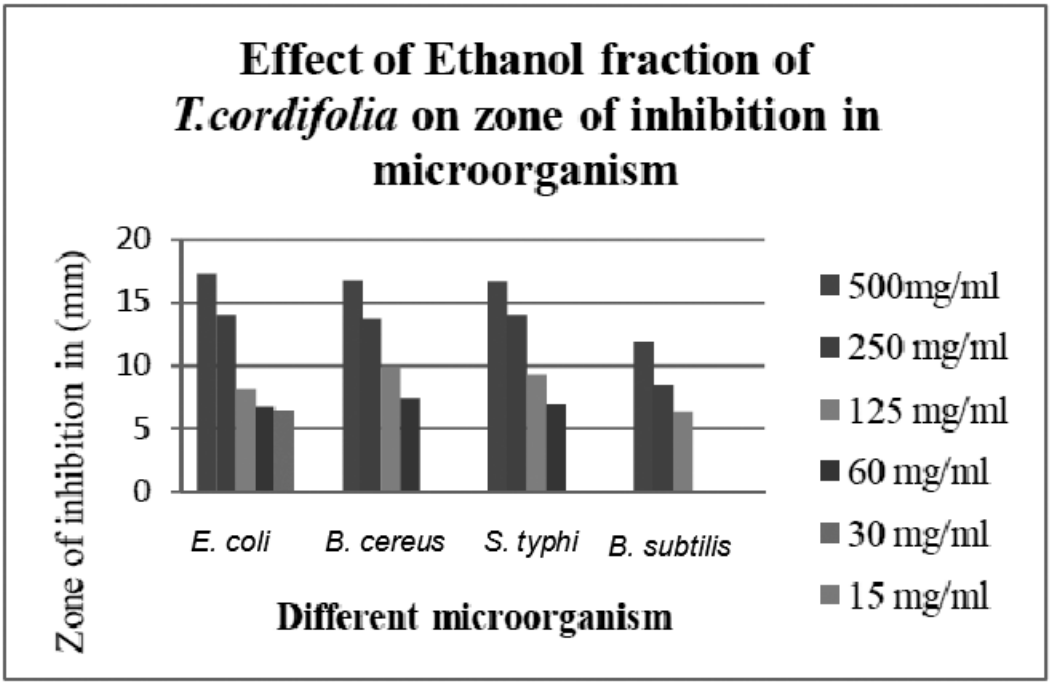



**Fig: 1.2: Antimicrobial activities of the hexane extract of Tionospora cordifolia plant in agar diffusion test**

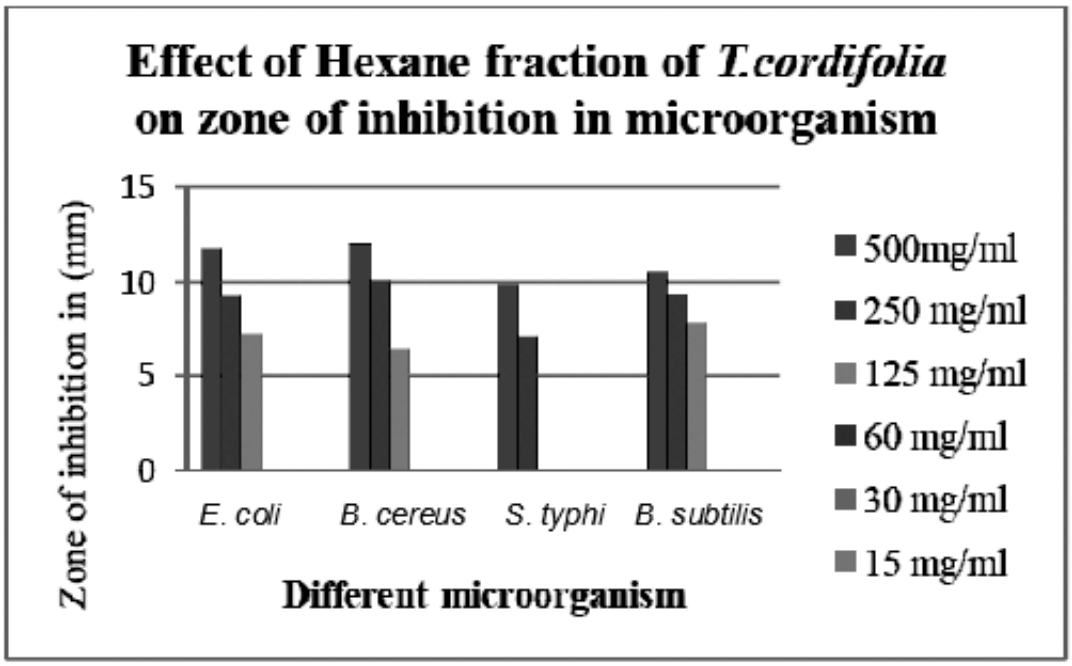
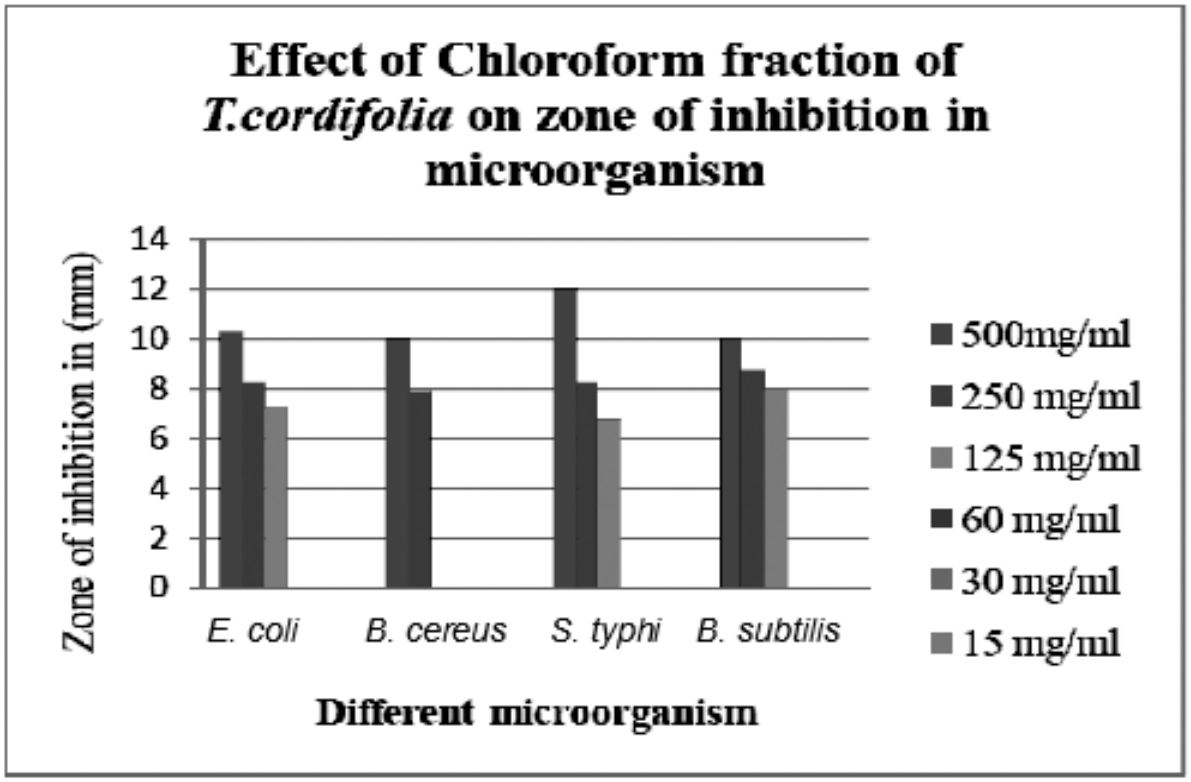


**Fig: 1.3: Antimicrobial activities of the chloroform extract of *Tionospora cordifolia* plant in agar diffusion test**




in case of agar well diffusion method, the hexane extract at 500 mg/ml of *Tinospora cordifolia* showed the maximum zone of inhibition (12.33±0.20 mm) against *Salmonella typhi* followed by *E. coli* (10.06±0.20 mm) and Bacillus cereus (10.03±0.26 mm). The extract of *Tinospora cordifolia* showed the minimum zone of inhibition (9.96±0.45 mm) against *Bacillus subtilis* (Figure 1.2).

In case of agar well diffusion method, the chloroform extract at 500 mg/ml of *Tinospora cordifolia* showed the maximum zone of inhibition (12.03±0.30 mm) against *Bacillus cereus* followed by *E. coli* (11.76±0.70 mm) and *Bacillus subtilis* (10.56 ± 0.28 mm). The extract of *Tinospora cordifolia* showed the minimum zone of inhibition (9.83±0.65 mm) against *Salmonella typhi* (Figure 1.3).

**CONCLUSION**

The demonstration of antimicrobial activity against tested bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity. The results of the study also support the traditional application of the plant and suggest that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs formulation for the treatment of gastroenteritis, typhoid fever and wound infections. In addition, there is need for the characterization and isolation of the active ingredients.

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