RESEARCH ARTICLE

In-vitro study showed that *Tinospora cordifolia* is having significant antibacterial effect against different human pathogenic strains in different dose dependent manner

Mohammad Nazrul Islam, Sharmin Rumi Alim

Islam MN, Alim SR. *In-vitro* study showed that *Tinospora cordifolia* is having significant antibacterial effect against different human pathogenic strains in different dose dependent manner. J Mic Bio Rep. 2022;5(5):58-61.

ABSTRACT

Now a day, antibiotics resistance becomes a serious health problem in the world. For this purpose, scientists are seeking effective natural medicine or antibiotic or metabolites to reduce the global health burden. They are given emphasis to conduct research on natural medicine because they know very well that nature is having all remedies of health problem. Plant based drugs are showing fewer side effects, less toxic and also cost effective. On study conducted it was found that the stems of *Tinospora cordifolia* were significantly efficient against *Escherichia coli, Proteus vulgaris, Enterobacter faecalis, Salmonella typhi, Staphylococcus aureus,* and *Serratia marcesenses*. Another study conducted found that *T. cordifolia* aqueous extracts have effective antimicrobial activity against all tested microorganisms. For example, the leaf aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *E. faecalis* (28 mm) and *S. typhi* (26 mm) at 50 mg/ml concentration. The *Tinospora cordifolia* is highly efficient against *Serratia marcescens, E. coli, Salmonella typhi, Streptococcus thermophilus, Fusarium oxysporium,* and *Aspergillus niger*, but that they had no effect on *Trichoderma reesei*. These plants can be a source of useful drugs but further studies are required to isolate the active component from the crude plant extract for proper drug development. That's why, we have selected a plant *Tinospora cordifolia* to explore the antibacterial activities against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholera* and *Klebsiella pneumonia*. It will help to combat infectious disease related mortality, morbidity, long-term consequences and also helps to play active role on antibacterial resistance.

Key Words: *Microbial resistance; Antibacterial activity; Zone of inhibition, Tinospora cordifolia; Human pathogenic strains of bacteria*

INTRODUCTION

Multi-Drug Resistant (MDR) becomes a global public health problem. It may be due to indiscriminate use or repeated use of antimicrobial medications without knowing their pharmacological properties. Even though, the cost of treating disorders with synthetic medications is higher. As a result, new infection-fighting tactics are needed to combat microbial illnesses [1,2]. It is proven that antimicrobial compounds can be found in abundance in medicinal plants. Only a small percentage of the plant species were phyto chemically analyzed from 250,000 species-500,000 species [3]. It is observed that *Tinospora cordifolia* (locally called Gulancha) is having antibacterial, anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-anti-malarial, hepatoprotective, and immunomodulatory activity. The study aimed to observe the in-vitro antibacterial activity of *Tinospora cordifolia* (Gulancha) extracts against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholerae* and *Klebsiella pneumonia* of human pathogenic strains.

LITERATURE REVIEW

It was observed that very low concentration of *Tinospora cordifolia* showed notable antimicrobial vulnerability against Gram-negative and Gram-positive microbes such as *Klebsiella pneumoniae* (ATCC 15380), *Escherichia coli* (ATCC 25922), *Micrococcus luteus* (ATCC 9341), *Streptococcus pneumoniae* (ATCC 12755), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778). Furthermore, aqueous extracts of TC plant species showed considerably larger inhibitory zone widths (20 mm-30 mm) than synthetic antibiotics against all seven bacterial strains (6-18). Certainly, the presence of certain chemical compounds in the plant is responsible for the aforesaid antimicrobial actions [4]. Jeyachandran et al. found that the stems of *Tinospora cordifolia* were significantly efficient against *Escherichia coli, Proteus vulgaris, Enterobacter faecalis, Salmonella typhi, Staphylococcus aureus*, and *Serratia marcesenses* [5]. Agarwal et al. found that *T. cordifolia* powder is efficient against *Streptococcus mutans* in one investigation. Its 2% concentration showed zone of hindrance of 19 mm, and the most intense antibacterial movement of *T. cordifolia* was observed. A zone of impediment of 28 mm was observed in 0.2% chlorhexidine, while no zone of restraint was observed in dimethylformamide. It was also found to have antibacterial properties against *S. mutans* [6].

Molla et al showed that the Minimum Inhibitory Concentration (MIC) of TC-1 against *Bacillus megaterium* and *Salmonella typhi*-A was reported to be 128 g/ml. The value of the medium lethal concentration, LC50 (9.34 g/ml), suggested that the chemical TC-1 had a strong hazardous impact [7].

Another study conducted by Nageswari found that *T. cordifolia* aqueous extracts have effective antimicrobial activity against *E. faecalis* (28 mm) and *S. typhi* (26 mm) at 50 mg/ml concentration [8].

Sandhu found that *Tinospora cordifolia* is highly efficient against *Serratia marcescens, E. coli, Salmonella typhi, Streptococcus thermophilus, Fusarium oxysporium*, and *Aspergillus niger*, but that they did not affect *Trichoderma reesei*. These plants can be a source of useful drugs but further studies are required to isolate the active component from the crude plant extract for proper drug development [9].

Tembeker found that *Tinospora cordifolia* was having significant antibacterial effects against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Shigella flexneri, Salmonella paratyphi, Salmonella typhimurium* and *Pseudomonas aeruginosa* [10].

It was found that *Tinospora cordifolia's* was significant antibacterial activity against three Gram-negative and two Gram-positive bacteria, namely *Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Staphylococcus aureus,* and *Bacillus subtilis*. The antibacterial activity was measured using Mueller-Hinton agar.

Tinospora cordifolia was very effective against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,Salmonella typhi*. The extract of TC1 has effective antimicrobial activity against all organisms, whereas the extract of TC2 has an inhibition zone on only a few species such as *Staphylococcus aureus*

Assistant Professor, Hamdard University Bangladesh.

Correspondence: Dr. Mohamamd Nazrul Islam, MD; Assistant Professor, Hamdard University Bangladesh, e-mail drnazrul@hamdarduniversity.edu.bd

Received: 13-August -2022, Manuscript No. PULJMBR-22- 5480; Editor assigned: 15- August -2022, Pre QC No. PULJMBR-22- 5480 (PQ); Reviewed: 31- August -2022, QC No. PULJMBR-22- 5480 (Q); Revised: 04- September -2022, Manuscript No. PULJMBR-22- 5480 (R); Published: 20-September -2022, DOI: 10.37532/puljmbr.2022.5(5).58-61.

(12 mm) and *Klebsiella pneumonia* (10 mm), *Salmonella typhi* (7mm).

The extract of *T. cordifolia* possesses a wide spectrum of anti-oxidant and antibacterial action against bacterial and fungal infections.

The study demonstrated the crude extract of Gulancha and purified components for antibacterial and cytotoxicity characteristics. Magnoflorin and Tembetarine were purified and recognized as alkaloids. Both the MIC (32 g/ml-64 g/ml) and MBC (128 g/ml-256 g/ml) of these compounds demonstrated excellent antibacterial activity against *Bacillus cereus* and *Staphylococcus aureus*. The MTT colorimetric test was used to assess the cytotoxicity activity of the isolated components and crude extract against the L929 and HEK293 cell lines. This showed weak cytotoxicity activity with IC50 values of 1162.24 µg/ml to 2290.00 µg/ml and 1376.67 µg/ml to 2585.06 µg/ml towards L929 and HEK293 cell lines, respectively.

Singh investigated the antibacterial efficacy of silver nanoparticles produced from TC against multidrug-resistant microorganisms. The zone of inhibition of silver nanoparticles from TC stems ranged from 10 mm ± 0.58 mm to 21 mm ± 0.25 mm. The MIC of AgNPs against Pseudomonas aeruginosa was reported to be 6.25 g/ml to 200 g/ml. TC silver nanoparticles have high antibacterial activity, making them a potentially useful antibacterial agent [11].

The antibacterial activity of TC has been demonstrated in clinical isolates, according to a study done by Shanthi and Nelson. Using urinary pathogens such as *E. coli, Klebsiella pneumoniae, Proteus vulgaris*, and *Pseudomonas aeruginosa*, researchers discovered that the ethanol extract of the TC leaf had a better inhibitory effect than the other extracts studied (aqueous and chloroform).

Plant extracts of *Tinospora cordifolia* (TC) have been shown to have potential against microbial diseases, according to a study conducted by Singh et al. *Tinospora cordifolia* extracts have been tested for antibacterial activity against a variety of Gram-positive and Gram-negative microorganisms. The antibacterial activity of TC stem extracts was tested against uropathogens, Escherichia coli, and Staphylococcus aureus, all of which cause UTIs. The disc diffusion method was used to show that the three solvent extracts of TC have varied antibacterial activity against both uropathogenic isolates, with ethanolic (highest)>methanolic (moderate)>aqueous (poor) in decreasing order [11].

It was demonstrated that the hydroalcoholic extract of the stem was generated by the maceration process *in vitro* antibacterial activity of TC. *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Enterobacter faecalis, Salmonella typhi*, all showed effective antibacterial action [12,13].

RESEARCH METHODOLOGY

The fresh *Tinospora cordifolia* stem was collected from the campus of Hamdard University Bangladesh and shed-dried. The taxonomic identity was confirmed by the Taxonomist of Pharmacy, Jahangir Nagar University. After getting the taxonomic confirmation, the plant was processed to form a fine powder. The fine powder was kept in an air-tight container and was used as a tested drug. Due to the absence of Soxhlet apparatus, the maceration process was followed to prepare extract. Pathogenic microorganisms were collected from the Centre for Advanced Research in Science (CARS). Antibacterial assay of *Tinospora cordifolia* was performed by Well diffusion method against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholerae* and *Klebsiella pneumonia* of human pathogenic strains.

Material required for the study

- Ethanol 96.4%
- Whatman No.1 (6 mm)
- Petri dish
- Muller Hinton Agar media
- Pathogenic strain (*Salmonella typhi*)
- Incubator
- Sterile glass rod
- Conical flask.

Preparation of stock solution

In this process, 80 gm of fine powder of *Tinospora cordifolia* are placed in a conical flask with 400 ml of 100% ethanol solvent & allowed to stand for 7 days with frequent agitation. Then the mixture was filtered with muslin cloth and after that, the extract was again filtered with Whatman Filter paper. After that, the extract was sent to the Food chemistry lab for rotary evaporation. The extract was evaporated by BUCHI's rotary evaporator at 78°C. Concentrated extract (200 MCL) was placed in a good disc for showing the antibacterial activity of *Tinospora cordifolia* against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholerae,* and *Klebsiella pneumonia*. The petri dish was kept in an incubator at 37°C. The result was observed after 24 hours and 48 hours.

Preparation of media

38 gm of Muller Hinton Agar media was dissolved in 1 L of distilled water. After that, the media was heated to dissolve, and keeping the dissolve the solution was for autoclaving. After that, the solution was placed in a sterile petri dish and kept for cooling then kept in the fridge.

Preparation of bacterial inoculation: Young cultures of *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholerae,* and *Klebsiella pneumonia* broth were prepared. Media containing Petri dishes were demoisturized in dry heat chamber for 2 min. After de-moisturization, the Petri dishes were inoculated *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholerae,* and *Klebsiella pneumonia* by cotton buds and kept in the incubator for 12 hours.

Maceration

In this process, solid ingredients are placed in a container with a tight stopper. The whole of the solvent was allowed to stand for at least 3 days (3 days -7 days) with frequent agitation until the soluble matter is dissolved. The mixture is then filtered with Muslin clothes and Whatman filter-No-1 respectively.

Test microorganisms: *Streptococcus pyogenes, Staphylococcus aureus, Salmonella typhi, Clostridium perfringens, Escherichia coli, Vibrio cholerae,* and *Klebsiella pneumonia*

Determination of zone of inhibition

The antibacterial activity of the extracts was tested *in vitro* using a good diffusion assay. A diluted (0.2 ml) bacterial culture of respective strains was poured in sterile 9 cm Petri plates containing 10 ml of Mueller Hinton agar medium and spread over agar plates using sterile glass L-rod, 200 µl of each extract was applied per well and was allowed to dry before being placed on to the top layer of the agar plates. The plates were incubated at 37°C for 24 hours. The experiments were carried out in triplicate and the average diameter of the zone of inhibitions was recorded. Results were expressed as mean ± standard deviation.

Mueller–hinton agar media

Mueller–Hinton agar is a microbiological growth medium that is commonly used for antibiotic susceptibility testing, specifically disk diffusion tests. It is also used to isolate and maintain Neisseria and Moraxella species [13].

It typically contains:

- 2.0-gm beef extract
- 17.5-gm casein hydrolysate
- 1.5-gm starch
- 17.0-gm agar
- 1 L of distilled water.
- pH adjusted to neutral at 25°C.

Preparation of mueller hinton agar

Suspend 38 gm of your Mueller Hinton agar powder (CM0337B) in 1 liter of distilled water.

- Mix and dissolve them completely.
- Sterilize by autoclaving at 121°C for 15 minutes.

Pour the liquid into the petri dish and wait for the medium to solidify. Be sure that you are preparing the agar in a clean environment to prevent any contamination.

Study design

In vitro antibacterial study

Bias was minimized by

Using Alcohol, demineralized sterile water, and blank

Data collection

The study was conducted in the microbiology laboratory, at the Institute of Nutrition and Food Sciences, University of Dhaka, and data was collected from the laboratory.

Data analysed and interpreted by

By measuring ZI.

RSESULTS

Table 1 represented that alcoholic extract of TC-20, TC-40 and TC-80 against *Vibrio cholerae* [ZI (mm): 14 mm,10 mm, 12 mm]; *Escherichia coli* [ZI(mm) :8,16,13], *Streptococcus pyogenes* [ZI(mm): 16 mm, 12 mm,16 mm], *Staphylococcus aureus* [ZI(mm) : 16 mm,12 mm,14 mm], *Salmonella typhi* [ZI(mm): 8 mm, 12 mm, 10 mm] and *Klebsiella pneumoni* [ZI(mm): 9 mm,13 mm, 12 mm] respectively (Figure 1).

Table 2 represents that the alcoholic extract of *Tinospora cordifolia*, TC-20, TC-40 and TC-80 against *Vibrio cholerae* [ZI(mm): 14 mm, 10 mm, 12 mm] ; *Escherichia coli* [ZI(mm): 8 mm,16 mm,16 mm], *Streptococcus pyogenes* [ZI(mm): 16 mm,12 mm,16 mm], *Staphylococcus aureus*[ZI(mm): 16,12,14), *Salmonella typhi* [ZI(mm): 8 mm, 12 mm and 10 mm] and *Klebsiella pneumonia* [ZI(mm): 9 mm,13 mm, 12 mm] respectively (Figure 2).

DISCUSSION

We found that alcoholic extract of TC-20, TC-40 and TC-80 against *Vibrio cholerae* [ZI (mm): 14 mm,10 mm, 12 mm]; *Escherichia coli* [ZI(mm): 8 mm,16 mm,13 mm]; *Streptococcus pyogenes* [ZI(mm): 16 mm,12 mm,16 mm]; *Staphylococcus aureus* [ZI(mm): 16 mm,12 mm,14 mm]; *Salmonella typhi* [ZI(mm): 8 mm, 12 mm, 10 mm] and *Klebsiella pneumoni* [ZI(mm): 9 mm,13 mm, 12 mm] respectively. One study conducted by Nagaprashanti et al showed that *Tinospora cordifolia* was very effective against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi*. The extract of TC1 has effective antimicrobial activity against all organisms, whereas the extract of TC2 has an inhibition zone on only a few species such as *Staphylococcus aureus* (12 mm) and *Klebsiella pneumonia* (10 mm), *Salmonella typhi* (7 mm). Both studies coincided with each other (Table 1).

TABLE 1

Antibacterial activity (Zone of Inhibition) of *Tinospora cordifolia* against different pathogenic strains after 24 hours

	Zone of Inhibition (mm) by Well diffusion method after 24 hours		
Pathogenic strain	**TC20**	**TC40**	**TC80**
Vibrio cholerae	14	10	12
Escherichia coli	8	16	13
Streptococcus pyogenes	16	12	16
Staphylococcus aureus	16	12	14
Salmonella typhi	8	12	10
Klebsiella pneumonia	9	13	12

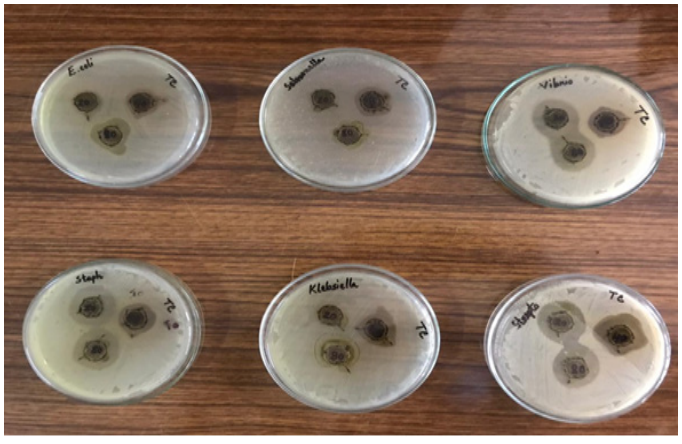

Figure 1) *Antibacterial activity of Tinospora cordifolia against different pathogenic strains after 24 hours*

TABLE 2

Antibacterial activity (Zone of Inhibition) of *Tinospora cordifolia* against different pathogenic strains after 48 hours

	Zone of Inhibition (mm) by Well diffusion method after 48 hours		
Pathogenic strain	**TC20**	**TC40**	**TC80**
Vibrio cholerae	14	10	12
Escherichia coli	8	16	13
Streptococcus pyogenes	16	12	16
Staphylococcus aureus	16	12	14
Salmonella typhi	8	12	10
Klebsiella pneumonia	9	13	12

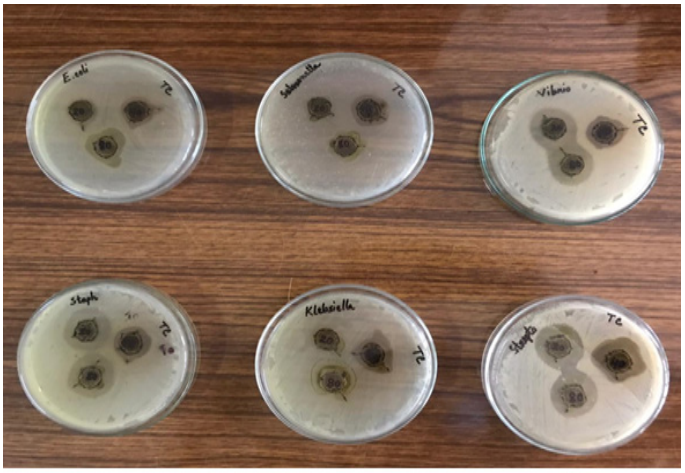

Figure 2) *Antibacterial activity of Tinospora cordifolia against different pathogenic strains after 48 hours.*

We observed that after 48 hours the alcoholic extract of *Tinospora cordifolia*, TC-20, TC-40 and TC-80 against *Vibrio cholerae* [ZI(mm): 14 mm, 10 mm, 12 mm]; *Escherichia coli* [ZI(mm): 8 mm,16 mm,16 mm], *Streptococcus pyogenes* [ZI(mm): 16,12,16], *Staphylococcus aureus*[ZI(mm): 16 mm,12 mm,14 mm), Salmonella *typhi* [ZI(mm): 8 mm, 12 mm and 10 mm] and *Klebsiella pneumonia* [ZI(mm): 9 mm,13 mm, 12 mm] respectively. One study conducted by Tembeker et al found that *Tinospora cordifolia* was having significant antibacterial effects against *Escherichia coli* [ZI(mm): 12 mm, 15 mm], *Staphylococcus aureus*[ZI(mm): 13, 15], , *Klebsiella pneumoni* [ZI(mm): 17, 15], *Salmonella typhi* [ZI(mm): 19 mm] [10]. Both studies result was showing significant antibacterial effects of *Tinospora cordifolia* though ZI were slightly higher than our study. It may be due to strain strength or different doses of tested drugs.

CONCLUSION

The alcoholic extract of *Tinospora cordifolia* is having a significant antibacterial effect against Staphylococcus, Streptococcus, and *E. Coli* strains. It has also effects on *Vibro cholera, Salmonella typhi,* and *Klebsiella pneumonia*.

REFERENCES

1. Dabur R, Gupta A, Mandal TK, et al. Antimicrobial activity of some Indian medicinal plants. African Journal of Traditional, Complementary and Alternative Medicines. 2007;4(3):313-8.
2. Sieradzki K, WU SW, Tomasz A. Inactivation of the methicillin resistance gene mecA in vancomycin-resistant Staphylococcus aureus. Microbial drug resistance. 1999;5(4):253-7.
3. Mahesh B, Satish S. Antimicrobial activity of some important medicinal plant against plant and human pathogens. World journal of agricultural sciences. 2008;4(5):839-43.
4. Upadhyaya R, Pandey RP, Sharma V, et al Assessment of the multifaceted immunomodulatory potential of the aqueous extract of Tinospora cordifolia. Research Journal of Chemical Sciences.2011.

5. Jeyachandran R, Xavier TF, Anand SP. Antibacterial activity of stem extracts of Tinospora cordifolia (Willd) Hook. f & Thomson. Ancient science of life. 2003;23(1):40.

6. Agarwal S, Ramamurthy PH, Fernandes B, et al. Assessment of antimicrobial activity of different concentrations of Tinospora cordifolia against Streptococcus mutans: An in vitro study. Dental research journal. 2019;16(1):24.

7. Molla AH, Zakaria MG, Molla MT, et al. Chemical investigation and microbial activity of Tinospora cordifolia miers. Journal of bio-science. 2012; 20:153-60.

8. Nageswari G, Reddy AH, Venkatappa B. Antibacterial activity of Tinospora cordifolia extracts on clinical isolates from HIV infected patients. Int J Curr Res. 2016;8(6):33072-7.

9. Sandhu A, Bhardwaj N, Gupta R, et al. Antimicrobial Activity and Photochemical Screening of Tinospora Cordifolia and Euphorbia Hirta. 2013.

10. Tambekar DH, Khante BS, Chandak BR, et al. Screening of antibacterial potentials of some medicinal plants from Melghat forest in India

11. Singh SS, Pandey SC, Srivastava S, et al. Chemistry and medicinal properties of Tinospora cordifolia (Guduchi). Indian journal of pharmacology. 2003;35(2):83.

12. Sharma A, Gupta A, Singh S, et al. Tinospora cordifolia (Willd.) Hook. F. & Thomson-A plant with immense economic potential. J. chem. pharm. Res. 2010;2(5):327-33.

13. Hendriksen RS. Susceptibility testing of Salmonella using disk diffusion. World Health Organization-WHO: USA. 2002:9.