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Original article

In vitro study of antimicrobial activity of *Tinospora cordifolia* (Thunb.) Miers plant extracts against selected clinical isolates

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Abstract

The present investigation was aimed to study the antimicrobial potential of *Tinospora cordifolia* (Thunb.) Miers commonly known as "Giloy" which is used by the herbal practitioners for curing several ailments. The antimicrobial potential was explored by using solvent extracts of *T. cordifolia* stem against clinical pathogenic isolates, *i.e., Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Yersinia pestis*. Extraction was carried out by cold percolation method, using various solvents, *viz*., chloroform, acetone, methanol and water. Among all, the chloroform and acetone extracts of *T. cordifolia* showed maximum activity against *S. aureus* (18.22 mm at 4 mg/ml). The minimum inhibitory concentration (MIC) of solvent extract of *T. cordifolia* was 6 mg in case of *S. aureus*. It was found that extremely small quantity of crude extract of *T. cordifolia* was sufficient to inhibit the growth of pathogenic microbes and it was quite comparable to the positive control used in many cases. These encouraging results can be taken further to purify and characterize the plant extracts and to check their antimicrobial activity against the pathogenic bacteria so as to utilize the pharmacological potential of the plant extract at higher scale after extensive R & D efforts.

Key words: *Tinospora cordifolia* (Thunb.) Miers, antimicrobial activity, dimethyl sulfoxide, MIC, herbal medication, medicinal plants

1. Introduction

Infection is the offensive and multiplication of microorganisms such as harmful bacteria, viruses and parasites that are not usually present within the body (Bhatt *et al*., 2013). Most of the population of developing countries depend on plant derived medicines for their primary healthcare needs as estimated by World Health Organization (WHO). It has been estimated that, nearly 2000 different microbes infect the human body and cause various types of diseases (Biradar, 2015). An infection may remain localized, or it may spread through the blood or lymphatic vessels to become systemic (Ananthnarayan and Paniker, 2009).

The antimicrobial activity of various herbal plant extracts has been extensively studied on many pathogenic microorganisms like *Staphylococcus aureus, E. coli, Listeria, Shigella* sp, *Proteus vulgaris, Salmonella typhi, Klebsiella, Streptococcus pneumoniae, Pseudomonas aeruginosa, Enterobacter faecalis, etc.* (Gordan and Antolic, 2001). Traditional plants have been played an imperative role in major healthcare needs (Anjaneyulu and Giri, 2018). Plant based natural product including extracts, essential oils, phytomolecules, *etc*., attract the pharmaceutical industry (Nooreen *et al*., 2018). Free radicals can damage cells, and may play a role in heart disease, cancer, inflammatory-bowel diseases and many other; however, antioxidants are the substances that may protect cells against the effects of free radicals. Medicinal plants contain variable chemical families and huge amounts of antioxidants (Ambawat and Khetarpaul, 2018).

Plants have an almost limitless ability to synthesize aromatic substances, mostly phenols or their oxygen-substituted derivatives (Thakur *et al*., 2017; Dogra, 2014). Most of them are secondary metabolites, of which less than 10% of the total (12,000) have been isolated (Mallikharjuna *et al*., 2007). The medicinal plant extracts contain various phytochemical compounds such as alkaloids, flavonoids, terpenoids, tannins and quinines which have inhibitory effect on numerous bacteria and fungi (Thakur, 2015; Rajeshwer, 2015; Jayasuriya *et al*., 1992). The scientific studies on various medicinal plants indicated that since their extracts contain components of therapeutic value; these can be used as medicines for curing various diseases which include cancer, diabetes and wounds (Nostro *et al*., 2000; Nayanabhirama, 2016; Reddy and Urooj, 2018). Moreover, in view of the easy availability of the herbal plants; successfully used in traditional system of medicine by the local inhabitants for curing several ailments based on the scientific data available in literature. Important medicinal plant species, *T. cordifolia* with reported medicinal values is found growing abundantly in Kangra region of Himachal Pradesh, India.

T. cordifolia is a glabrous, succulent and climbing shrub, native to India, also found in Burma and Sri Lanka. The reference of medicinal uses of *T. cordifolia* to treat diseases like vatarakta (gouty arthritis) and daha (burning sensation) is found in various Ayurvedic texts. It has been traditionally used in various formulations for the treatment of rheumatoid arthritis (Badar *et al*., 2005). Therefore, the present study was undertaken with major focus on the evaluation and

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validation of medicinal properties of *T. cordifolia* which has been used by local herbal practitioners /Vaidya's for successfully curing several ailments due to the presence of various important biologically active compounds which can be used for the synthesis of traditional herbal medicines. The antimicrobial potential of *T. cordifolia* stem extracts was evaluated against various clinical pathogenic strains.

2. Material and Methods

2.1 Collection of plant material

The samples of stem of *T. cordifolia* were collected from Dharamshala area of Kangra district, Himachal Pradesh, India during the months of March and April 2014 due to the probability of high quantity of desired phytochemicals during this season as also reported earlier. The identification of plant sample was done in the Department of Biosciences, Himachal Pradesh University, Shimla, and HFRI (ICFRE), Shimla. The voucher specimen is also deposited in the department. The plant material was cleaned with fresh water, dried under shade about 2 to 4 weeks and stored under air-tight containers until further use.

2.2 Clinical isolates

The clinical isolates (*Staphylococcus aureus, Bacillus cereus, Pseudomonas aeruginosa, Streptococcus pneumoniae* and *Yersinia pestis*) were procured from Department of Microbiology, Indira Gandhi Medical College and Hospital (IGMC), Shimla, Himachal Pradesh, India.

2.3 Selected clinical isolates and antibiotics

Table 1: Clinical isolates and antibiotics used

Bacteria	Antibiotics used
Staphylococcus aureus	Tetracycline
Bacillus cereus	Chloramphenicol
Pseudomonas aeruginosa	Gentamycin
Streptococcus pneumoniae	Erythromycin
Yersinia pestis	Erythromycin
DMSO was used as negative control for bacterial isolates	

2.4 Preparation of plant extracts

The sample was ground to powdered form using pestle, mortar and grinder. The powder was stored in air tight container until further use. Cold percolation method was used for the preparation of plant extracts as per the method detailed by Rosenthaler (1930). Different solvents, *i.e.*, chloroform, acetone, methanol and water were used for extraction and the crude plant extracts after evaporation were stored at – 4°C in refrigerator for further analysis. Dried stem powder of *T. cordifolia* was added into the respective solvent in the ratio of 1:10 in 250 ml flask and kept on a rotary shaker (150 rpm) for 48 h. at 35°C. The extract was filtered through Whatman No.1 filter paper and allowed to evaporate under room temperature. The residual plant material from the first extraction was further transferred in the next solvent.

2.5 Stock solution

The stock solution of plant extracts was prepared by using 10% dimethyl suloxfide **(**100 µl DMSO diluted to 900 µl distilled water) universal solvent in such a way that final concentration comes to be 100 mg/ml.

2.6 Determination of antimicrobial potential

The effect of different plant extracts on clinical isolates was measured by agar well diffusion method. The agar well diffusion method was used to assess the antimicrobial activities. The crude plant extracts were allowed to diffuse out into the agar medium and interact in a plate freshly spreaded with the test microorganism. Mueller Hinton agar medium was poured into autoclaved petri plates and allowed to solidify under aseptic conditions. 20 µl of 24 h old culture of the each test organism was spread separately on the specific plates and labeled properly. Six well of 6 mm diameter were gently bored using sterile borer. Different volumes of plant extracts were added to the respective well. Known antibiotics (5 mg/ml) as positive control and DMSO as negative control were loaded in the respective wells. Plates were incubated at 37°C for overnight incubation. The resulting zones of inhibition appearing as uniform circular areas around the wells indicated the absence of bacterial growth which was taken as a standard for the determination of antimicrobial potential (Cappuccino and Sherman, 1999). The minimum inhibitory concentration (MIC) of the plant extract required to inhibit the growth of microorganism was analyzed by Resazurin dye method using plant extract (McNicholl *et al*., 2006). Resazurin, a non-fluorescent and non-toxic blue colored oxidation-reduction indicator becomes pink and fluorescent when reduced to resorufin by oxidoreductases within viable cells. Resorufin is further reduced to colorless and non-fluorescent hydroresorufin. The MIC is the only most effective extracts, *i.e.*, acetone and chloroform were determined against all pathogenic microbes using decreasing concentrations of extracts in the range of 2 mg/ml to 8 mg/ml. The MIC of each extract was determined after 24 hour of incubation at 37°C and any color change from purple to pink or colorless was taken as positive. All experiments were conducted in triplicates.

3. Results

3.1 Effect of different concentrations of various extracts of *T. cordifolia* on pathogenic isolates

3.1.1 Chloroform extract

The chloroform extract exhibited a good antimicrobial activity against all the tested pathogenic isolates as detailed in Figure 1. The extract was found highly effective against *S. aureus* (13.33 mm and 15.24 mm at 3 mg/ml and 4 mg/ml), closely related to positive control, *i.e.*, gentamycin 19 mm, followed by *Y. pestis* (13.23 mm at 4 mg/ml) and least effective against *Y. pestis* (7.22 mm at 2 mg/ml).

3.1.2 Acetone extract

The effect of different concentrations of acetone extract of *T. cordifolia* was observed and zones of inhibition were recorded against each test pathogens as presented in Figure 2. The extract showed maximum activity against *S. aureus* (18.22 mm at 4 mg/ml) which was greater than positive control gentamycin (*i.e.*, 14 mm), followed by *S. pneumoniae* (14.27 mm and 16.24 mm at the 3 mg/ml and 4 mg/ml) which was quite comparable to positive control,

i.e., erythromycin 18 mm. The inhibitory effect of acetone extract on *P. aeruginosa* (15.21 mm), *B. cereus* (16 mm) and *Y. pestis* (16.23 mm) was quite comparable to positive control.

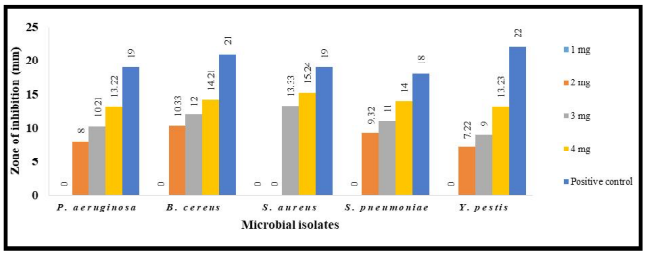

Figure 1: Antibacterial activity of chloroform extract of *T. cordifolia* against various pathogenic strains.

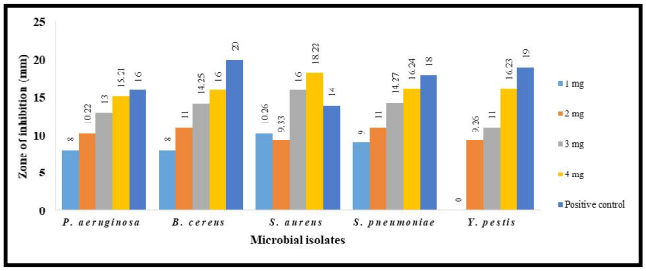

Figure 2: Antibacterial activity of acetone extract of *T. cordifolia* against various pathogenic strains.

3.1.3 Methanol extract

The effectiveness of methanol extract of *T. cordifolia* was recorded against almost all the pathogenic strains except *P. aeruginosa* and *S. pneumoniae* as presented in Figure 3. The extract showed maximum inhibitory activity against *S. aureus* at all concentrations, *i.e.*, 16 mm at 4 mg/ml which was closely related to positive control, *i.e.* (18 mm). In case of *Y. pestis* (13 mm at 4 mg/ml), the inhibitory effect was greater than positive control, *i.e.*, tetracycline (12 mm). The extract was least effective against *B. cereus* (7.33 mm at 1 mg/ml).

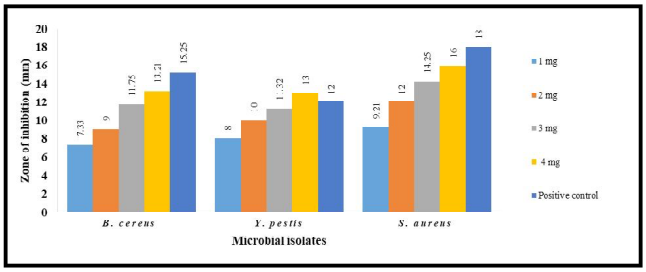

Figure 3: Antibacterial activity of methanol extract of *T. cordifolia* against various pathogenic isolates.

3.1.4 Aqueous extract

The aqueous extract of *T. cordifolia* showed maximum antimicrobial potential against almost all the tested pathogens as presented in Figure 4. The highest inhibitory effect was observed against *S. aureus* (15.24 mm at 4 mg/ml), followed by *Y. pestis* (14.21 mm at 4 mg/ml). The inhibitory effect was comparable to positive control against *P. aeruginosa* (12 mm at 4 mg/ml). The extract was least effective against *S. pneumoniae* (8 mm at 2 mg/ml).

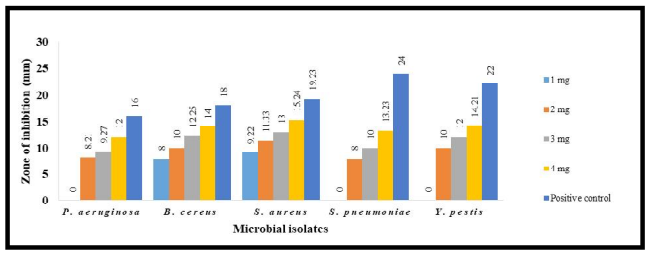

Figure 4: Antibacterial activity of aqueous extract of *T. cordifolia* against various pathogenic strains.

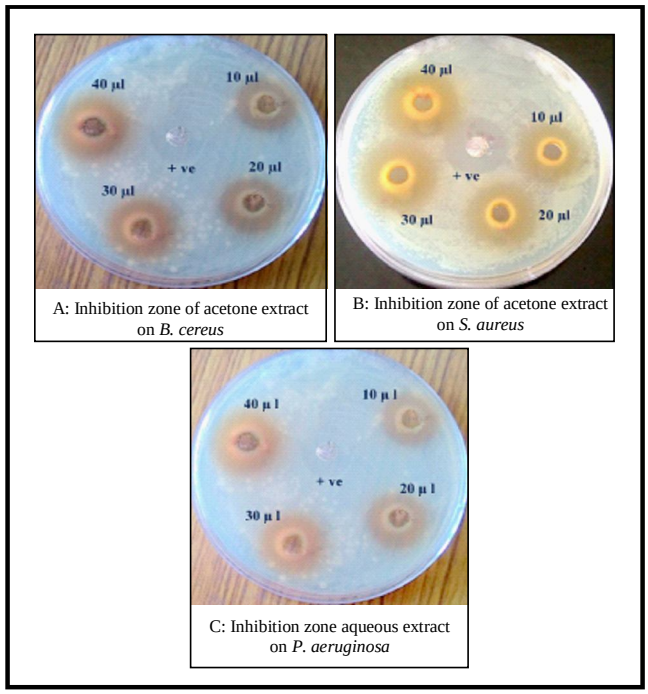

Figure 5: Effect of acetone and aqueous extract of *T. cordifolia* against pathogenic microorganism.

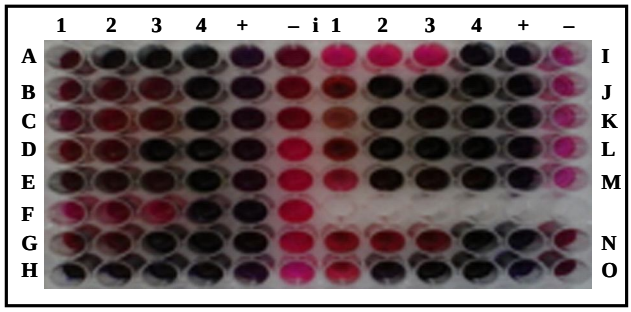

Figure 6: Minimum inhibitory concentration (MIC) of stem extracts of *T. cordifolia* against pathogenic bacteria.

3.2 Minimum inhibitory concentration (MIC) of chloroform and acetone extract of *T. cordifolia*

The minimum quantity/vol. needed to inhibit or to kill the pathogenic strains. MIC was carried out using chloroform and acetone extract of *T. cordifolia* as these extracts were more effective among the others. The minimum concentration of chloroform and acetone

extracts of *T. cordifolia* required to inhibit the growth of *S. aureus* was 6 mg/ml whereas in case of *B. cereus* and *S. pneumoniae* MIC was 4 mg/ml.

Table 2: MIC of chloroform and acetone extract of *T. cordifolia* against pathogenic isolates

Sr. No	Conc. of plant extract (mg/ml)	*P. aeruginosa*	*S. aureus*	*B. cereus*	*S. Pneumoniae*	*Y. pestis*
1	2	-	-	-	-	-
2	4	+	-	-	-	+
3	6	+	-	+	+	+
4	8	+	+	+	+	+
MIC (mg/ml)		2	6	4	4	2

– = no bacterial growth; + = bacterial growth

4. Discussion

The present investigation was aimed to evaluate the *in vitro* antimicrobial activity of solvent extracts of *T. cordifolia* stem against Gram-positive (*Streptococcus pneumoniae, Bacillus cereus* and *Staphylococcus aureus*) and Gram-negative (*Yersinia pestis* and *Pseudomonas aeruginosa*) pathogenic bacterial strains. It has been reported that this important medicinal plant mainly contains alkaloids, glycosides, steroids, aliphatic compound, essential oils, mixture of fatty acids, polysaccharides, tannins, phenolic compounds, and other aromatic compounds that produce a definite physiological action on human body including anti-inflammatory, antitumor and antidiabetic activities (Dutta and Panse, 1962; Betoni *et al*., 2006).

Of various solvent extracts of *T. cordifolia*, chloroform and acetone extracts exhibited good activity against all the pathogenic bacterial strains *S. aureus, B. cereus, P. aeruginosa, S. pneumoniae* and *Y. pestis* used in the present study. Regarding comparative efficacy, the chloroform extract of *T. cordifolia* showed significant activity against S. aureus (15.24 mm at 4 mg/ml). In a study conducted by Nagaprashanthi *et al*. (2012), the extract of TC1 was found effective against all the organisms (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas* spp. *Aspergillus niger*, *Aspergillus fumigates*, *mucor* spp. and *Pencillium*), whereas the extract of TC2 exhibited inhibition zones on *S. aureus* (12 mm), *K. pneumonia* (10 mm), *Pseudomonas* spp. (8 mm), *A. niger* (6 mm), *A. fumigates* (8 mm) and *mucor* spp. (12 mm). Sharma and Prajapati (2016) have also reported the antibacterial potential of *T. cordifolia* against *E. coli* and *S. aureus*.

Acetone extracts of *T. cordifolia* was found most effective against *S. aureus* (18.22 mm at 4 mg/ml). Aneja (2004) has also reported better activity of the acetone extract against *Staphylococcus aureus* (12 mm). In the present study, maximum inhibitory effect of methanol extract of *T. cordifolia* was found at 4 mg/ml against *S. aureus* (16 mm) and *B. cereus* (13.21 mm). The study conducted by Nageshwari *et al*. (2016) evaluated the antibacterial activity of leaf and stem extract of *T. cordifolia* against *Escherichia coli, Enterococcus faecalis, Streptococcus pneumoniae, Salmonella typhi* and *Staphylococcus aureus*. The leaf extract of *T. cordifolia* exhibited maximum zone of inhibition against *E. faecalis* (28 mm) and *S. pneumonia* (26 mm) at 50 mg/ml concentration.

The aqueous extract of *T. cordifolia* stem was also quite effective against *S. aureus* (15.24 mm at 4 mg/ml) which is quite comparable to positive control. Singh *et al*. (2015) have observed that aqueous extract of *T. cordifolia* exhibited activity against *E. coli* (0.16 ± 0.033 cm). Mishra *et al*. (2013) have also reported the inhibition of aqueous extract of *T. cordifolia* against *K. pneumoniae* (11.33 ± 0.58), *E. coli* (19.00 ± 1.00) and *Pseudomonas* spp (14.67 ± 0.58). The phytochemical variation and comparative medicinal value of *T. cordifolia* has been reported to vary with geographical locations and seasons (Jeyachandran *et al*., 2003). The organic and aqueous extracts of *T. cordifolia* have been reported to have potential as a source of useful phytochemical compounds for the pharmaceutical industry (Devprakash *et al*., 2011).

In the present study, minimum inhibitory concentration of chloroform and acetone extract of *T. cordifolia* required to inhibit the growth of *S. aureus* was 6 mg/ml. Verma *et al*. (2011) have found stem extract of *T. cordifolia* effective in the range of 0.625-1.25 mg/ml against *E. coli, Listeria, Pseudomonas aeruginosa* and *Bacillus*. The results obtained indicated the supremacy of *T. cordifolia* extracts which inhibited growth of all bacterial strains tested and that too at much lower concentrations. The findings reported in the present work can help in development of new therapeutic agents through plant/ plant extract after further R&D efforts especially w.r.t. utility of purified plant constituents and application of biotechnological principles.

5. Conclusion

It is well known fact that *T. cordifolia* "Giloy" is an important medicinal plant which has been extensively used in Ayurveda, Siddha, Unani and other medicinal systems for centuries together and is also being used successfully in rural as well as urban areas by several herbal practitioners especially in the developing countries. The plant contains a number of phytoconstituents, which provide medicinal value to the plant. The results showed that the solvent extract of the stem possess strong antimicrobial activity and can serve as source of new therapeutic agents against various pathogenic bacteria. This can be one of the better options for the pharmacologists in their research for new drugs from natural sources. On comparison with other plants it was found that a very less quantity of crude extract of *T. cordifolia* was enough to inhibit growth of pathogenic microbes which was also quite comparable to the positive control used in many cases. The efficacy can be improved by phytochemical analysis and purification of the bioactive compounds and using these in disease cure with the blend of modern scientific interventions. These encouraging results can be taken further to purify and characterize the plant extracts and to check the antimicrobial activity against the pathogenic bacteria.

Conflict of interest

We declare that we have no conflict of interest.

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