Original Research Article

Antibacterial and antifungal effects of indigenously grown stem and leaf extracts of *Tinospora cordifolia* - A preliminary study

Damel Lakshmi U[1], Smiline Girija AS[2], Priyadarshini G[1], Prasanna CC[3], Kayalvizhi E[4], Chandrasekhar M[4]

[1]Department of Physiology, [2] Department of Microbiology, [3]Central Research Laboratory, Meenakshi Ammal Dental College and Hospital, Maduravoyal, Chennai, [4]Meenakshi Medical College Hospital and Research Institute, Enathur, Kanchipuram

Abstract

Background: Therapeutic modalities have inclined towards alternative medicine involving usage of herbs and plants as a source to treat chronic ailments. They are considered to be safe, efficacious and are less toxic when compared to chemically synthesized agents. In this context, *Tinospora cordifolia (T. Cordifolia)* tropical vine indigenous to India, Myanmar and Srilanka, has been immensely used in traditional medicine and Ayurvedic preparations. The emergence of drug resistant organisms and increasing drug toxicity are two major obstacles of the current treatment methods. Seeking an alternative drug with less toxicity and more efficacy is a safer option for society in the current scenario. Several studies have therefore been performed to elucidate the antimicrobial activity of *T. Cordifolia*. **Aim:** The present study was designed to demonstrate the antimicrobial property of methanolic, ethyl-acetate and hexane extracts of indigenously grown stem and leaf extracts of *T. Cordifolia* against common pathogenic bacteria and fungi. **Materials & Methods:** The antibacterial and antifungal properties of the crude extracts were assessed by agar well diffusion method. The minimum bactericidal concentration [MBC] and minimum inhibitory concentration [MIC] were determined using microbroth dilution method followed by confirmation by Microbial Spot Checker board method. **Results:** The crude extracts of the plant showed promising activity against Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae and Pseudomonas aeruginosa. **Conclusion:** This study revealed that the methanol, ethyl acetate and hexane extract of the leaf and stem of indigenously grown *Tinospora cordiflora* had promising activity against common pathogenic bacteria. Although the antimicrobial activity of this plant is established and has produced promising results when replicated in-vitro, further toxicity studies are warranted to provide concrete evidence about the safety of its extracts.

Key words: agar well diffusion method, antibacterial activity, Tinospora cordifolia

Corresponding author
Damel Lakshmi U, Lecturer (Research scholar), Department of Physiology, Meenakshi Ammal Dental College and Hospital, Alapakkam road, Maduravoyal.
Telephone: +91 9094117103, Email: damel_lakshm@yahoo.co.in

Introduction

Emergence of multidrug resistant organisms (MDR) and selective toxicity towards synthetic drugs has hampered the process of antimicrobial therapy in recent times. A suggestive alternative to the current treatment method is to employ bioactive compounds which are more efficacious and with less or no toxic effects on the host tissues. Also, the bioactive material should be easily metabolised by the system to minimize toxic effects. Plant sources possess a plethora of active ingredients which can be utilized to treat several diseases. One such plant known to possess a wide array of bioactivity is *Tinospora*

cordifolia (T. cordifolia) which is an important drug in Ayurvedic medicine.[1] It is defined as a Rasayana drug in Ayurveda, which is recommended to enhance resistance and promote longevity and is given the name "Amritha" which signifies its use for revitalization and as an anti-stressor.[1]

T. cordifolia has been reported to possess anticancer,[2] antidiabetic,[3] anti-inflammatory,[4] antipyretic, hepatoprotective, immunomodulatory,[5] and diuretic activities. It has also been found to minimize toxic side effects during cancer chemotherapy. Alkaloids, glycosides, flavonoids, steroids, terpenoids, saponins, tannins, lignans are the major constituents of this plant species, which has enriched its role in the folk medicine.[6] Digestive disorders, loss of appetite, fever, dysentery, urinary diseases,[7] viral hepatitis,[8] anaemia are also cured using herbal formulation from *T. cordifolia.*

Antibacterial activity of *T. cordifolia* extract has been reported against Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia, Proteus vulgaris, Salmonella typhi, Shigella flexneri, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacter aeruginosa.[9,10,11] Extract from *T. cordifolia* has also shown improved phagocytic activity invivo.[12] The root extract of this plant has shown to exhibit anti-HIV activity by reducing the eosinophil count, stimulation of B cells, macrophages and polymorpho nuclear leucocytes.[13,14]

The synthesis of metabolites from plants though controlled by genetic factors are influenced by age, growth phase, site, season etc., and environmental factors contribute immensely to the quantity and quality of active principles and secondary metabolites procured from medicinal plants. Hence, the current study aimed to analyse the antimicrobial activity of *T. cordifolia* collected from in and around Chennai city, Tamil Nadu.

Materials and methods

The study was done in Meenakshi Ammal Dental College, Chennai after obtaining the required ethical clearance. The antibacterial and antifungal effect of *Tinospora cordifolia (T. Cordifolia)* was assessed by using different extracts like methanol, ethyl acetate and hexane solvents.

[i] Plant source: Fresh plants of *T. cordifolia* were collected from various parts of Chennai and the species was identified and certified by an eminent botanist. The stem and leaf portions were washed in plain water, shade dried for 30 days and was blended into fine powder using an electric blender.[5] The powders were stored in air-tight sterile containers until use for the study.

[ii] Preparation of the crude extracts: Crude solvent extraction of *T. cordifolia* was achieved by dissolving 25gms of the powdered leaf and stem in 75 ml of methanol, ethyl acetate and hexane [1:3 w/v] separately in sterile brown bottles and was allowed to stand for 3 days at 37^{o}C in an orbital shaker. The extracts were then filtered and evaporated using rotary evaporator and were weighed in a digital balance. The crude extracts were further re-suspended in dimethyl sulphoxide [DMSO], to a final concentration of 100mg/ml and was stored in 4^{o}C until further studies.

[iii] Antimicrobial bio-assay: The antibacterial and antifungal properties of the crude extracts were assessed by agar well diffusion method[6] against the test organisms viz., Staphylococcus aureus [ATCC No.25923], Escherichia coli [ATCC No. 25922], Klebsiella pneumonia [ATCC No.10031], Pseudomonas aerugenosa [ATCC No.27853], Enterococcus faecalis [ATCC No.29212] and Candida albicans [ATCC No.10231].

The bacterial inoculums were prepared and were adjusted to 0.5 McFarland turbidity standards. Lawn cultures of the test organism were made onto the surface of sterile Mueller Hinton agar [MHA] for the bacterial pathogens and onto the surface of Saboraud's dextrose agar [SDA] for the yeast. Wells were punctured in the agar using agar borer, and 50µl of the crude extract of 100 mg/ml concentration were added into each specified well. Erythromycin [30µg], ciprofloxacin [10µg] and amphotericin B [10 U] were included as positive controls for gram positive, gram negative and yeast respectively. DMSO was included as negative control. All the plates were incubated at 37^{o}C/24 hrs/aerobically.

Zone of inhibition was measured using Hi-media antibiotic measuring scale and were recorded. The antimicrobial efficacy was graded based on the zone diameter as high activity (> 15 mm), moderately active (10-14 mm), trace activity (5-9 mm) and no activity (< 4 mm).[7]

[iv] Determination of MBC and MIC values: The minimum bactericidal concentration [MBC] and minimum inhibitory concentration [MIC] were determined using microbroth dilution method.[8] Briefly, serial dilutions of the crude extracts from methanol stem extract and ethyl acetate leaf extract were made in a 96 well micro titre plate with DMSO. The dilution factor was 100, 50, 25, 12.5, 6.25 and 3.125 mg/ml. To each dilution 100 µl of the culture broths of the test organism were added in their respective wells and the plate was incubated at 37^{o}C for 24 hrs. After incubation the spectrophotometric analysis was performed and the OD values were recorded.

The MIC value was also confirmed by Microbial Spot Checker board method[9] where 3 µl of each dilution was spotted onto Mueller Hinton Agar plates and incubated at 37^{o}C for 24 hrs. After incubation the spot showing the complete absence of microbial growth indicates the MBC value and the spot with less visible growth indicates the MIC value.

Statistical analysis: The results obtained were statistically analyzed using SPSS software version 16, for calculating the mean of the zones of inhibition on tested microorganisms. Mann-Whitney U test was done to compare the zones of inhibition of the *T. cordifolia* extracts with the control antibiotic and antifungal drug.

Table 1: Antimicrobial and antifungal effect of the crude extracts of the stem of *Tinospora cordifolia* against the test organisms

Test organisms under study	Stem extracts [Mean ± S.D]			Control		
	M	EA	H	ERY	CIP	AMPH
Staphylococcus aureus [ATCC No: 25923]	19 ± 1.0	11.3 ± 0.57	12 ± 2.0	24	-	-
Enterococcus faecalis [ATCC No: 29212]	11 ± 1.0	NA	10.3 ± 1.5	24	-	-
Klebsiella pneumoniae [ATCC No: 10031]	NA	NA	NA	-	25	-
Escherichia coli [ATCC No: 25922]	NA	NA	NA	-	25	-
Pseudomonas aeruginosa [ATCC No: 27853]	13 ± 0.5	NA	NA	-	26	-
Candida albicans [ATCC No: 10231]	NA	NA	NA	-	-	26

Zone of inhibition expressed in mm, NA = No activity, Me = Methanol, EA = Ethyl acetate, H = Hexane, Ery = Erythromycin, Cip = Ciprofloxacin, Amph = Amphotericin B,

Results

This study designed to demonstrate the antimicrobial property of methanolic, ethyl-acetate and hexane extracts of indigenously grown stem and leaf extracts of *T. Cordifolia* against common pathogenic bacteria and fungi revealed the following results:

The antimicrobial activity of stem extract of *Tinospora cordifolia* showed significant effect on S.aureus (P>0.05) when compared with K. pneumoniae, E. coli and C. albicans. The activity was observed with stem extract against E. faecalis and P. aeruginosa when compared with other extracts. The activity obtained due to Methanol extract showed promising antibacterial activity when compared with other extracts as shown in Table 1.

As shown in Table 2, the anti microbial activity of the leaf extract showed moderate activity against S. aureus and P. aeruginosa with minimal activity against the yeast whereas other extracts did not show any action against C. albicans. The three extracts did not show any activity against E. coli and E. faecalis.

The minimum bactericidal concentration [MBC] and minimum inhibitory concentration [MIC] values were deduced as 100, 50 and 25 µg/ml for the tested bacteria whereas the MIC and MBC were recorded as 25 and 50 µg/ml for the yeast C. albicans (Table 3). MIC and MBC was recorded at a low concentration of 25 µg/ml against C. albicans and P. aerugenosa revealing it as an effective antibacterial and antifungal drug of choice.

Table 2: Antimicrobial and antifungal effect of the crude extracts of the leaf of *Tinospora cordifolia* against the test organisms

Test organisms under study	Leaf extracts [Mean ± S.D]			Control (mm)		
	M	EA	H	Ery	Cip	Amph
Staphylococcus aureus [ATCC No: 25923]	NA	10.3 ± 1.5	11.6 ± 0.5	24	-	-
Enterococcus faecalis [ATCC No: 29212]	NA	NA	NA	24	-	-
Klebsiella pneumonia [ATCC No: 10031]	NA	10.6 ± 0.5	NA	-	25	-
Escherichia coli [ATCC No: 25922]	NA	NA	NA	-	25	-
Pseudomonas aeruginosa [ATCC No: 27853]	9.6 ± 1.1	12	NA	-	26	-
Candida albicans [ATCC No: 10231]	NA	10 ± 1.0	NA	-	-	26

Zone of inhibition expressed in mm, NA = No activity, Me = Methanol, EA = Ethyl acetate, H = Hexane, Ery = Erythromycin, Cip = Ciprofloxacin, Amph = Amphotericin B,

Table 3: MBC and MIC determination of the stem and leaf extracts of *Tinospora cordifolia*

Test organisms under study	Methanolic extract of stem [100 µg / ml]		Ethyl acetate extract of leaf [100 µg / ml]	
	MBC	MIC	MBC	MIC
Staphylococcus aureus	100	50	100	50
Enterococcus faecalis	50	25	NT	NT
Klebsiella pneumonia	NT	NT	100	50
Escherichia coli	NT	NT	NT	NT
Pseudomonas aeruginosa	100	50	50	25
Candida albicans	NA	NA	50	25

NT = not tested

Discussion

The herbal plants are an important source of new chemotherapeutic agents. The active principles of these herbal plants are responsible for therapeutic use in human beings. Many studies are available on either antibacterial or antifungal effect separately or in combination using different drugs. In the present study, we studied the effect of the methanol, ethyl acetate and hexane extract of leaf and stem of T. cordiflora against common pathogenic bacteria and fungi**.** Jeyachandran *et al.,* performed a comparative antimicrobial study on three different crude extracts of *T. cordifolia* viz., aqueous, ethanol and chloroform.[19] The study reported that ethanolic extracts exhibited significant antibacterial activity against P. vulgaris, E. coli, with a moderate activity observed against E. faecalis.[19] Additionally, the stem extracts with chloroform showed moderate inhibition against E.coli, P. vulgaris, E.faecalis.[19] The results obtained from the present study are in good agreement with the above mentioned report. Both the studies have utilized polar solvents which have shown promising antimicrobial activity. Different doses of methanolic extract viz., 10 and 20 mg of this plant were found to have antimicrobial activity against Bacillus subtilis (MTCC8), E.coli (MTCC1), Staphylococcus aureus (MTCC98), Salmonella typhi (MTCC737). Out of all pathogens tested S.aureus showed a greater zone of inhibition of 13 and 18 cm respectively for the doses studied.[20] The antibacterial activity of *T. Cordifolia* was also tested against dental pathogens such as S.aureus (MTCC 1144), Streptococcus mutans (MTCC 890), Streptococcus salivarius (MTCC 1938), Lactobacillus acidophilus (MTCC 447) and Streptococcus sanguinis (ATCC 10556).[21] Methanolic extract was found to be effective against all the dental pathogens included with maximum activity against Streptococcus sanguinis (23mm) and Streptococcus salivarius (17mm).[21]

A recent (2013) study by Gawai *et al.,* investigated the antibacterial and antifungal activities of the ethanolic extracts of *T. cordifolia, Phyllanthus niruri and Abrus precatorious* against the pathogenic E. coli and fungi Aspergillus niger and Epidermophyton floccosum by the filter paper disc method.[22] The results obtained indicated that the leaf extract *T. cordifolia* showed highest antimicrobial activity against E. coli at a concentration of 100mg/ml, thus elucidating the potential effect of *T. cordifolia* extract on gram negative organisms.[22] Another study by

Shanthi *et al.,* in 2014, demonstrated that the ethanolic extracts of *T. cordifolia* leaf showed maximum antifungal activity against Candida albicans which has been attributed to berberine content.[23] Aqueous stem extract of *T. cordifolia* was tested against E. coli O78 strain, isolated from a natural case of coli bacillosis in poultry. Broth dilution assay was performed to demonstrate the antibacterial activity of T.cordifolia.[24] The maximum activity of extract was observed at a dilution of 1:32 and minimum inhibitory concentration was found to be 1:64 dilutions.[24]

The ethyl acetate extracts have greater effect than methanol and hexane extracts which could be due to the solubility of active compounds from plant in ethyl acetate than other solvents. Among stem and leaf extraction of all the solvents, ethyl acetate leaf extract and methanolic stem extract has shown more prominent zone of inhibition. This results correlates with the earlier studies and aided in further assessment of MIC and MBC determination[25].

Elucidating the molecular pathway leading to bioactivity of the extracts is warranted to convert the crude extract into a pharmacological compound. The synergistic and antagonist effects of the extract with other compatible herbal compounds may be experimented on to provide a more efficacious drug. In vitro plant tissue culture methods can be used to propagate the plant to study the effective period of production of secondary metabolites. Thus, this pilot study opens new avenues to embark on the research arena involving medicinal plants.

Conclusion

This study revealed that the methanol, ethyl acetate and hexane extract of the leaf and stem of indigenously grown *Tinospora cordifolia* had promising activity against common pathogenic bacteria. Further toxicity studies are warranted to provide concrete evidence about the safety of its extracts.

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Conflicts of interest: Nil

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