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IN VITRO STUDY OF ANTIMICROBIAL PROPERTIES OF *TINOSPORA CORDIFOLIA* (GUDUCHI)

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ABSTRACT

The aim of the present study was to evaluate the antimicrobial activity of *Tinospora cordifolia* extracts against three standard ATCC strains of *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa*. The efficacy of extract was measured in terms of Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and zone of inhibition (mm). Antimicrobial activity of aqueous as well as ethanolic extracts were tested in vitro using standard microbroth dilution method with double dilution, against ATCC strains of *E.coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* for determination of MIC. MBC values were determined by subculture on standard solid media. The Kirby Bruer's Disk Diffusion Method was then used to observe the zones of inhibition to a range of concentration at MIC values with other broad spectrum antibiotics. Both aqueous and ethanolic extract of *Tinospora cordifolia* showed activity against standard ATCC strains of *E.coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro at very high concentrations. Further studies are required to explore the antimicrobial efficacy of *Tinospora cordifolia* against clinical isolates.

KEY WORDS: Herbal extract, antibiotic, micro-organisms, resistance.

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INTRODUCTION

Antibiotic discovery was a critical moment in medical history that has revolutionized medicine in many regards, and myriad lives have been saved consequently. But it is unfortunate that, the injudicious use of these drugs has been conjoined with the prompt development of resistant strains of microorganisms. Medical professionals are now cautioning of a reappearance of the pre-antibiotic era. A recent database registers the existence of more than 20,000 potential resistance genes of nearly 400 different types.[1] Antimicrobial resistance is fostered due to indiscriminate use of antimicrobial drugs by unduly prolonged or inadequate disease treatment. Because of high level of antimicrobial drug resistance, it is mandatory to use expensive drugs which may not be affordable by majority of patients in developing countries like India. [2] Thus, antibiotic discovery, their mechanisms of action, and modes of resistance have been dynamic research topics in academics as well as in the pharmaceutical industry. [3] As natural products, plants may provide a natural source of antimicrobial drugs that may give novel or lead compounds that may serve as an alternative to the existing antimicrobials.[4] Many modalities of medicines like Allopathy, Ayurveda, Homoeopathy and Unani employ plant products for treatment of different ailments. Currently, Ayurveda considered as a vital system of medicine, is getting the global appreciation and having comparatively less toxic substances.[5] In the last few decades, a number of plant species were studied for retrieving promising antimicrobials for therapeutic utility that subsequently turned into an intrinsic part of elementary health care in many parts of world including India. Plants are rich source of wide variety of secondary metabolites viz. tannins, terpenoids, alkaloids, and flavonoids, which have profound antimicrobial properties. [6] Hence, plants that possess strong antimicrobial potential against pathogens are considered as a valuable source of medicinal compounds and are likely to have lesser side effects in comparison with modern medicines. *Tinospora cordifolia* is a widely used shrub in folk and ayurvedic systems of medicine. The chemical constituents reported from this shrub belong to different classes such as alkaloids, diterpenoid lactones, glycosides, steroids, phenolics, aliphatic compounds and polysaccharides.[7] These chemical constituents are reported to have activity in diabetes, arthritis, allergic conditions, leprosy, malaria, cancer and also reported to possess hepatoprotective, immunomodulatory and activity against many infections.[8,9,10] Chemical analysis of this plant have revealed a number of protoberberine and aporphine alkaloids having antiprotozoal and antimicrobial properties.[11,12] Thus, the present study was undertaken to evaluate in vitro antimicrobial activity of this herbal preparation.

MATERIALS AND METHODS

Whole plant powder of *Tinospora cordifolia,* was purchased from Himalaya Drug Company, Bangalore (Batch no. - F130001G, Manufacturing date - March 210, Expiry date - April 2013). This herbal preparation was further processed for obtaining different types of extracts as decoction and ethanolic extracts.

Extraction by Decoction

Ten grams of the powdered plant material of *Tinospora cordifolia* was macerated in sterile distilled water for 24 hours (1:16 times), then boiled and reduced to 1/4th of its quantity. Further, the extract was filtered using Whatman filter paper no. 1 and clear filtrate was obtained. This extract was further diluted and used for analysis of antimicrobial activity.[13,14]

Ethanolic extraction

Fifty grams of whole plant material of *Tinospora cordifolia* was macerated for 24 hours in 75% ethanol and subjected to cold percolation in a percolator. Extraction was done by continuous percolation method till the collected fluid become colorless. The collected extract was reduced to 1/4th in rotary vacuum evaporator at

40^0 C and subjected to evaporation at 40^0 C in water bath till a semisolid extract was obtained. A yield of 50% was obtained after the method of extraction that was further diluted and used for assessment of its antimicrobial activity.

MIC & MBC

In vitro antimicrobial activity of *Tinospora cordifolia* was assessed for its Minimum Inhibitory Concentration (MIC), Minimum Bacteriocidal Concentration (MBC), and suppressive activity on standard strains using standard microbiological techniques. Antimicrobial activity of aqueous as well as ethanolic extracts were tested in vitro using standard broth dilution method with double dilution, against American Type Culture Collection (ATCC) strains of *E.coli* 25922, *Staphylococcus aureus* 25923 and *Pseudomonas aeruginosa* 27853 for determination of MIC in Non-inhibitory liquid media (NHLM). MBC was then determined by subculture from NHLM on Non inhibitory plating media (NHPM). The Kirby Bruer's Disk Diffusion Method was then used to observe the zones of inhibition to a range of concentration at MIC values against three standard ATCC strains. Standard ATCC strains were inoculated in peptone water to achieve a turbidity of 0.5 McFarland unit. Lawn culture was made on Muller Hilton Agar (MHA) plate with sterile swab sticks. Wells of 2 mm diameter were cut in MHA plate. Five such wells were used for extract in doubling dilution with one well for negative control of distilled water and broad-spectrum antibiotic disk as positive control. The diameter of zone of inhibition to various dilutions of extract were measured and compared.

RESULTS

In the present study, both the aqueous extract and ethanolic extract of *Tinospora cordifolia* showed activity against standard ATCC strains of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The MIC, MBC values and zone of inhibition are shown in table 1 and table 2 for aqueous extract and ethanolic extract of *Tinospora cordifolia* respectively

The MIC and MBC values for aqueous extract were found to be 200 mg/ml and 400 mg/ml whereas values for ethanolic extract were found to be 250mg/ml and 500mg/ml respectively for all the three control strains.

In case of *Staphylococcus aureus* the zone of inhibition was 18 mm for 800 mg/ml and 9mm and 6 mm for 400 and 200 mg/ml respectively when aqueous extract was used whereas for ethanolic extract it varied from 9mm, 5 mm and 3mm for 1gm/ml, 500 mg/ml, 250mg/ml respectively. Best results were seen for *Staphylococcus aureus, Pseudomoas aeruginos*a and then *Escherichia coli*.

Table 1

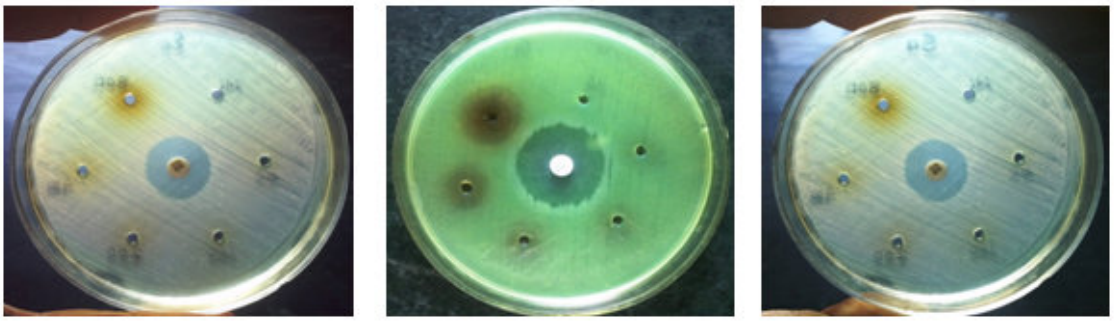
Aqueous extract of Tinospora cordifolia showing sensitivity at different concentrations

In-vitro Antimicrobial activity Parameters	Concentration in mg/ml																	
	800mg/ml			400mg/ml			200mg/ml			100mg/ml			50mg/ml			25mg/ml		
	EC	SA	PA	EC	SA	PA	EC	SA	PA	EC	SA	PA	EC	SA	PA	EC	SA	PA
Visible Turbidity (Growth) in drug containing NILM	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
Demonstration of Growth from drug containing NILM to NIPM	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Suppression of growth in millimeter around wells (Diameter) filled with drug on MHA	7	18	7	4	9	4	3	5	3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

EC - Escherichia coli, SA - Staphylococcus aureus, PA - Pseudomoas aeruginosa, NILM – Non-Inhibitory Liquid Medium, NIPM – Non-Inhibitory Plating Medium, Minimum Inhibitory concentration (MIC= Visible Turbidity (Growth) in drug containing NILM), Minimum Bactericidal concentration (MBC= Demonstration of Growth from drug containing NILM to NIPM), Zone of Inhibition = Suppression of growth in millimeter around wells (Diameter) filled with drug on Muller Hilton Agar (MHA), Y=Positive finding, N=Negative finding.

Figure1

Petri dishes showing zone of inhibition with aqueous extract of Tinospora cordifolia.



Escherichia coli ***Staphylococcus aureus*** ***Pseudomonas aeruginosa***

Table2

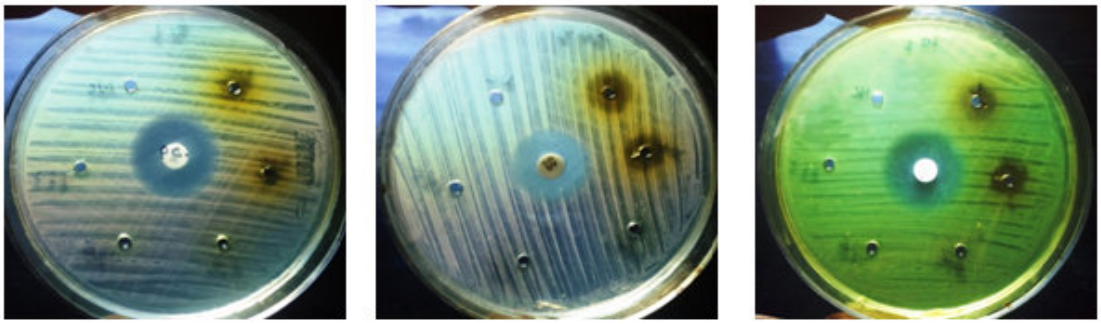
Ethanolic extract of Tinospora cordifolia showing sensitivity at different concentrations

In-vitro Antimicrobial activity Parameters	Concentration in mg/ml																	
	1000mg/ml			500mg/ml			250mg/ml			125mg/ml			62.5mg/ml			31.25mg/ml		
	EC	SA	PA	EC	SA	PA	EC	SA	PA	EC	SA	PA	EC	SA	PA	EC	SA	PA
Visible Turbidity (Growth) in drug containing NILM	N	N	N	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y
Demonstration of Growth from drug containing NILM to NIPM	N	N	N	N	N	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Suppression of growth in millimeter around wells (Diameter) filled with drug on MHA	6	9	6	4	5	4	1	3	1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

EC - Escherichia coli, SA - Staphylococcus aureus, PA - Pseudomoas aeruginosa, NILM – Non-Inhibitory Liquid Medium, NIPM – Non-Inhibitory Plating Medium, Minimum Inhibitory concentration (MIC= Visible Turbidity (Growth) in drug containing NILM), Minimum Bactericidal concentration (MBC= Demonstration of Growth from drug containing NILM to NIPM), Zone of Inhibition = Suppression of growth in millimeter around wells (Diameter) filled with drug on Muller Hilton Agar (MHA), Y=Positive finding, N=Negative finding.

Figure2

Petri dishes showing zone of inhibition with ethanolic extract of Tinospora cordifolia.



Escherichia coli ***Staphylococcus aureus*** ***Pseudomonas aeruginosa***

DISCUSSION AND CONCLUSION

Plant-derived medicine has been part of conventional health care in most parts of the world for thousands of years. The successful use of any therapeutic agent is compromised by the potential development of resistance to that compound that are used in the treatment of bacterial, fungal, parasitic, and viral infections. [1] Majority of the population in developing countries depends on medicinal products.[15] *Tinospora cordifolia* family Menispermaceae known as 'Amrita' or 'Guduchi' is widely used by folk Aurvedic system and Rasayanas to improve the immune system and body resistance against infections.[16] The present study was undertaken to study the antimicrobial properties of *Tinospora cordifolia* in vitro. Antimicrobial activity of aqueous as well as ethanolic extracts were tested in vitro using standard broth dilution method with double dilution, against ATCC strains of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* for determination of MIC. MBC was determined by subculture on non-inhibitory plating media. The Kirby Bruer's Disk Diffusion Method was then used to observe the zones of inhibition. According to results of present study, the zone of inhibition was observed for very high concentrations of both aqueous and ethanolic extract of *Tinospora cardifolia*. The undiluted extract and first dilution have shown bacteriocidal activity but subsequent dilutions of both ethanolic and aqueous extract have not shown any bacteriocidal activity. There was decreasing zone of inhibition for double dilution till two such dilutions. Best results were seen for *Staphylococcus aureus*, then for *Pseudomoas aeruginosa* followed by *Escherichia coli*. Similar studies in the past have documented the antimicrobial activity of different extracts of *Tinospora cordifolia* in various micro-organisms.[9,15,17,18,19,20,21] In a study by Upadhyay et al (2011), it was observed that in presence of *Tinospora cordifolia* chloroform extract maximum inhibition zone diameter was obtained as 25mm in *K. pneumoniae*, 26mm in *Escherichia coli* and *Staphylococcus aureus*, 27mm in *M. luteus* at 320µg concentration whereas in *Tinospora cordifolia* acetone extract maximum inhibition zone diameter was obtained as 25mm in *K. pneumoniae*, *M. luteus* and *L. acidophilus*.[9] In another study by Jeyachandran et al (2003), it has been reported that ethanolic extracts exhibited significant antibacterial activity against *Proteus vulgaris*, *Escherichia coli* while moderate activity was observed against *Enterobacter faecalis*. [15] In yet another study by Singh et al (2012) have concluded that maximum antibacterial activity of hot and cold methanol extracts was exhibited against *Staphylococcus aureus* when compared with standard drug Ciprofloxacin.[19] The results of present study are in accordance with the results of the study by Shivam Singh et al. Further studies are required to explore antimicrobial properties of *Tinospora cordifolia* especially in clinical isolates and/or studies in experimental animals in vivo.

Conflict of Interests: Declared none.

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