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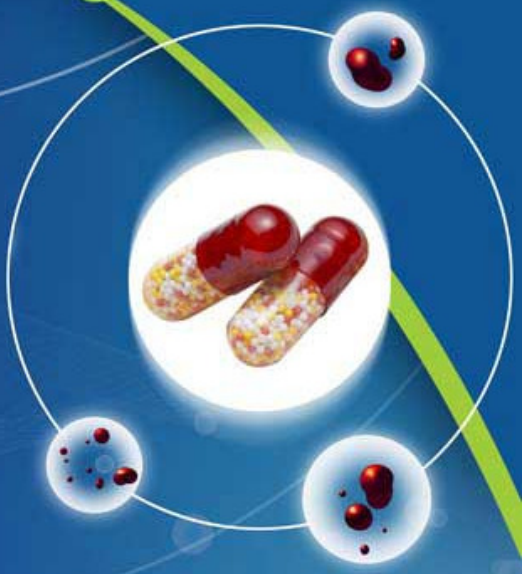

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Research Article

IN VIVO ANTIMICROBIAL ACTIVITY OF INDIAN MEDICINAL PLANT *TINOSPORA CORDIFOLIA* USING SERIAL TUBE DILUTION TECHNIQUE

Sharma A.*, Batra A.

Department of Botany, University of Rajasthan, **Jaipur. Rajasthan, India**

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ABSTRACT

The present study designed for the methanolic extracts of different in vivo (root, stem, leaf and bark) plant samples of Tinospora cardifolia (family: Menispermaceae) were screened for their antimicrobial activity against Escherichia coli, E. cloacae, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, A. niger, A. flavus, A. solani, R. stolonifer and F. oxisporum by using the MIC "Serial tube dilution technique" and agar well diffusion method. It is clear from the results that, the extracts of these plants acts as a good source of antibiotics against various bacterial and fungal pathogens tested and exhibited broad spectrum of antimicrobial activity. The largest zone of inhibition was observed for methanolic leaf extract against Staphylococcus aureus (15.5 ± 0.14 mm) and minimum was observed in bark against F. oxisporum and Pseudomonas aeruginosa (6.0 ± 0.45mm). Minimum inhibitory concentration (MIC) may be defined, as the lowest concentration of antimicrobial agent requires to inhibit the growth of organism. The maximum MIC values were recorded in stem and leaf i.e 128 µg/ml against Bacillus cereus, Staphylococcus aureus, A. niger, A. flavus, A. solani and R. stolonifer.The study suggest further research regarding the pharmacological investigations of this plant and also support the continued sustainable use of these plants in traditional systems of medicine.

Key words*: Tinospora Cordifolia, Plant extracts, Antibacterial, MIC, Antimicrobial activity.*

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization [1] medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [2].

*Correspondence author
Abhimanyu Sharma
Senior Research Scholar
Plant tissue culture and Biotechnology lab
Department of Botany, University of Rajasthan, Jaipur. India
E.mail:- abhsbiotech87@gmail.com

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. In the last few years, a number of studies have been conducted in different countries to prove such efficiency [3-8]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to an alarming increase in the incidence of new and reemerging infectious diseases and development of resistance to the antibiotics in current clinical use [9]. The screening of plant extracts has been of great interest to scientists in the search for new drugs for greater effective treatment of several diseases [10]. Therefore, plant extracts and phytochemicals with known

significance in therapeutic treatments [11-13].With the advancement in science and technology, remarkable progress has been made in the field of medicine with the discoveries of many natural and synthetic drugs. Antibiotics are undeniably one of the most important therapeutic discoveries of the 20th century that had effectiveness against serious bacterial infections. However, only one third of the infectious diseases known have been treated from these synthetic products. This is because of the emergence of resistant pathogens that is beyond doubt the consequence of years of widespread indiscriminate use, incessant misuse and abuse of antibiotics [14-16]. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs [17-18]. The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Plants are complex chemical storehouses of undiscovered biodynamic compounds, many of which serve as plant defense mechanisms against invasion by micro-organisms, insects and herbivores that can provide valuable sources of natural antibacterial agents [19-20]. The active principles isolated from plants appear to be one of the important alternatives when compared with many sub standard orthodox synthetic medicines because of their less or no side effect and better bioavailability. Plant extracts have been studied against pathogens for years for assays to detect new and previously undiscovered antimicrobials from plant sources [21-22].

In the present investigation, *Tinospora cordifolia a* indigenous plant species from India have been screened for their antimicrobial activities. It is a large glabrous ascending shrub belongs to family Menispermaceae and known as Giloy in Hindi. The leaves are membranous and cordate. It is used as a blood purifier and anti-infectious agent. It is also used for the treatment of jaundice, rheumatoid arthritis, diabetes, gout, viral hepatitis, arthopathies and general weakness.

Hence, in the present investigation, the antimicrobial potential of methanolic extracts of different plant parts (root, stem, bark, leaves) of *Tinospora cardifolia* have been evaluated against common fungal and bacterial pathogens.

MATERIALS AND METHODS

Collection of Plant material

Different plant parts (root, stem, bark and leaves) of *Tinospora cardifolia* were obtained from nursery of botany department, University of Rajasthan, jaipur. All the parts were washed with sterile water, dried in shade, finely powdered and stored in air tight bottles.

Preparation of plant extract

25 g of each air-dried powder of *Tinospora cardifolia* were immersed in 100 ml of methanol separately in a conical flask. It was incubated at room temperature for 48 hour at 150 rpm in an orbital shaker. The suspension was passed through Whatman No.1 filter paper, and concentrated to dryness at 40 ° C in hot air oven. All extracts were stored at 4°C in a refrigerator until screened.

Test Organisms

Both gram positive [*Bacillus cereus* (MTCC 4317), *Enterobacter cloacae* (7097), *Staphylococcus aureus* (MTCC 3160)] and gram negative bacteria [(*Escherichia coli, Pseudomonas aeruginosa,*)] as well as fungal [(*Aspergillus niger* (MTCC 282), *R. stolonifer* (MTCC 2591), *Aspergillus flavus* (MTCC 2456), *Alternaria solani* (MTCC 2101), *F.* oxisporum(MTCC 6659)] strains were used for the experiment which were collected as pure cultures from the Institute of Microbial Technology (IMTECH), Chandigarh, India.

Preparation of inoculums

Stock cultures were maintained at 4 °C on nutrient agar (HiMedia) and Potato dextrose agar (HiMedia) respectively for bacteria and fungi slants. Active cultures for experiments were prepared by transferring a loopful of culture to 10 mL of nutrient broth and PDA then incubated at 37 °C for 24 hours for bacterial proliferation and 25 °C for 48 hours for fungal activity.

Media preapration and its sterilization

For agar well diffusion method antimicrobial susceptibility was tested on solid (Agar-agar) media in petri plates. For bacterial assay nutrient agar (NA) (40 gm/L) and for fungus PDA (39 gm/L) was used for developing surface colony growth. The minimum inhibitory concentration (MIC), were determined by Serial tube dilution technique. The suspension culture, for bacterial cells growth was done by preparing 2% Lauria Broth (w/v), and for fungus cells growth, 2.4% (w/v) PDB (Potato dextrose broth) was taken for evaluation. All the media prepared was then sterilized by autoclaving the media at (121°C) for 20 min.

Preparation of plates

Prepared agar was then allowed sterilize and then allow cooling till 50 °C in a water-bath. Pouring of about 20 ml agar into pre-labeled sterile Petri dishes was made. They were then permitted to set at room temperature and were dried so that no drops of moisture remain on the surface of the agar.

Agar well diffusion

Agar well bioassay was employed for testing antimicrobial activity in different plant parts of *Tinospora cardifolia*. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Wells (6mm) were made in the agar plate with a sterile cork borer. The plant extract (100 µl) was introduced into the well and the plates were incubated at 37 °C for 24 hours for bacterial and 25 ºC for 48 hours for fungal activity.

The antimicrobial activity of the plant extract was determined by measuring the diameter of the inhibition zone. Controls contained only methanol which served as a negative or a positive control, respectively. The antimicrobial assay for each of the extracts against all microorganisms tested was performed in triplicates.

MIC "Serial tube dilution technique"

MIC was determined using "Serial tube dilution technique" In this technique the tubes of broth medium, containing graded doses of compounds are inoculated with the test organisms. After suitable incubation, growth will occur in those tubes where the concentration of compound is below the inhibitory level and the culture will become turbid (cloudy). Therefore, growth will not occur above the inhibitory level and the tube will remain clear.

Procedure

- Twelve test tubes were taken, nine of which were marked 1,2,3,4,5,6,7,8,9, and the rest three were assigned as T_M(medium), T_{MC}(medium + compound) and T_{MI}(medium + inoculum)
- 1 ml of nutrient broth medium was poured to each of the 12 test tubes.
- These test tubes were cotton plugged and sterilized in an autoclave for 15 lbs /sq. inch pressure.
- After cool 1 ml of the sample solution was added to the 1st test tube and mixed well and then 1ml of this content was transferred to the 2nd test tube.
- The content of the second test tube was mixed well and again 1 ml of this mixture was transferred to the 3rd test tube. This process of serial dilution was continued up to the 9th test tube.
- 10 µl of properly diluted inoculum was added to each of 9 test tubes and mixed well.
- To the control test tube T_{MC}, 1 ml of the sample was added, mixed well and 1 ml of this mixed content was discarded to check the clarity of the medium in presence of diluted solution of the compound.
- 10µl of the inoculum was added to the control test tube T_{MI}, observed the growth of the organism in the medium used.
- The control test tube T_M, containing medium only was used to confirm the sterility of the medium.
- All the test tubes were incubated at 37 °C for 18 hours.

OBSERVATION AND RESULTS

In the present investigation, the inhibitory effect of crude methanolic extracts of different plant parts from *Tinospora cardifolia* were evaluated against both fungicidal and bacterial strains (gram positive and gram negative). *In vivo* antimicrobial activity was determined using agar well diffusion method and Serial tube dilution technique in Table 1 to 5. The activity was quantitatively assessed on the

basis of inhibition zone and their minimum inhibitory concentration (MIC).

Measurement of inhibition zone diameter

Antibacterial activity: Antimicrobial activity of crude extract was also confirmed by agar well diffusion method and inhibition zone diameters were measured in different plant parts (methanolic extract) of *Tinospora cordifolia* (Table 1). The largest zone of inhibition was observed for methanolic leaf extract against *Staphylococcus aureus* (15.5 ± 0.14 mm) and minimum was observed in methanolic stem extract *B. cereus* (10.8 ± 0.43 mm). Where as in stem extract the largest zone of inhibition was observed against *Staphylococcus aureus* (11.0 ± 0.45mm) and minimum was observed in *B. cereus* (8.0 ± 0.13). However, in methanolic bark and root extract the highest zone of inhibition was found against *Staphylococcus aureus* (12.6 ± 0.52) and (15.0 ± 0.32 mm) respectively but minimum zone in bark and root (6.0 ± 0.79mm and 8.8 ± 0.13mm) against *P.aeruginosa*
and *E. coli*, respectively.

Antifungal activity:
The largest zone of inhibition was observed for methanolic leaf and root extract against *A. niger* (15.0 ± 0.43 mm and 14.6 ± 0.56 mm), respectively. Whereas, minimum antifungal activity was observed in methanolic leaf and root extract against *A. flavus and R. stolonifer* (9.3 ± 0.52 mm and 8.0 ± 0.29), respectively. However, in stem and bark extract the largest zone of inhibition was observed against *A. niger* (9.2 ± 0.35mm and 9.0 ± 0.19mm) and minimum was observed in *A. flavus* (6.6 ± 0.36) and *F. oxisporum* (6.0 ± 0.45 mm), respectively.

Determination of MIC

Methanolic Bark extract:

The first sign of growth of microorganisms *viz. B. cereus* and *E. coli* were observed in the test tube (no. 5) at a concentration of 32 µg/ml. So the MIC value of the methanolic root extract (where no bacterial growth was observed) was found to be 64 µg/ml (test tube no. 4) against *B. cereus* and *E. coli*. However, *E. cloacae* and *P. aeruginosa* growth of these micro-organisms were observed in the test tube no. 6 at a concentration 16 µg/ml (test tube no. 6), indicating that the MIC value of the methanolic root extract was 32 µg/ml against *E. cloacae* and *P. aeruginosa.* Furthermore, the growth *S. aureus* were observed in the test tube no. 4 at a concentration 64 µg/ml, indicating that the MIC value of the methanolic root extract was 128 µg/ml (Table 2).

On considering the antifungal activity, the growth of *A. niger R. stolonifer, A. flavus* and *F. oxisporum* were observed in test tube no. 4 (64 µg/ml) representing MIC at 128 µg/ml. Moreover, *A. solani* the growth of these micro-organisms were observed in the test tube no. 5 at a concentration 32 µg/ml, indicating that the MIC value of the methanolic bark extract was 64 µg/ml (test tube no. 4) (Table 2).

Methanolic Root extract:

The first sign of growth of organisms *B. cereus, E. cloacae* and *S. aureus* were observed in the test tube (no. 6) at a concentration of 16 µg/ml. So the MIC value of the methanolic root extract (where no bacterial growth was observed) was found to be 32 µg/ml (test tube no. 5) against *B. cereus, E. cloacae* and *S. aureus*. Whereas, in case of *E. coli* and *P. aeruginosa* the growth were observed in the test tube no. 4 and 7 at a concentration 64 µg/ml and 8 µg/ml, signifying that the MIC value of the methanolic root extract was 128 µg/ml and 16 µg/ml (test tube no. 3 and 6), respectively (Table 3).

However, in *R. stolonifer and A. solani* the growth of these micro-organisms were observed in the test tube no. 7 at a concentration 8 µg/ml, indicating that the MIC value of the methanolic stem extract was 16 µg/ml (test tube no. 6) against *R. stolonifer and Alternaria solani*. Furthermore, *A. niger*, *A. flavus* and *F. oxisporum* the growth of these micro-organisms were observed in the test tube no. 6 at a concentration 16 µg/ml, indicating that the MIC value of the methanolic root extract was 32 µg/ml (test tube no. 7) (Table 3).

Methanolic Stem extract:

The first sign of growth of micro organisms *B. cereus*, *Staphylococcus aureus* were observed in the test tube (no. 4) at a concentration of 64 µg/ml. So the MIC value of the methanolic stem extract (where no bacterial growth was observed) was found to be 128 µg/ml (test tube no. 3) against *B.*

cereus, *S. aureus*. In case of *E. cloacae* and *E. coli* the growth of these micro-organisms were observed in the test tube no. 5 at a concentration 32 µg/ml, representing that the MIC value of the methanolic stem extract was 64 µg/ml (test tube no. 4) against *E. cloacae* and *E. coli.* However, in *P. aeruginosa* the growth of these micro-organisms were observed in the test tube no. 6 at a concentration 16 µg/ml, indicating that the MIC value of the methanolic leaf extract was 32 µg/ml (test tube no. 5) against *P. aeruginosa* (Table 4).

Moreover, in *R. stolonifer and F. oxisporum* the growth of these micro-organisms were observed in the test tube no. 6 at a concentration 16 µg/ml, indicating that the MIC value of the methanolic stem extract was 32 µg/ml (test tube no. 7). In addition to this, *A. flavus* and *A. solani* the growth of these micro-organisms were observed in the test tube no. 4 at a concentration 64 µg/ml, signifying that the MIC value of the methanolic stem extract was 128 µg/ml (test tube no. 3). Furthermore, in *A. niger aeruginosa* the growth of these micro-organisms were observed in the test tube no. 5 at a concentration 32 µg/ml, indicating that the MIC value at 64 µg/ml (Table 4).

Methanolic Leaves extract: In *E. cloacae, E. coli* and *P. aeruginosa* the first sign of growth was observed in the test tube (no. 6) at a concentration of 16 µg/ml. So the MIC value of the methanolic leaf extract (where no bacterial growth was observed) was found to be 32 µg/ml (test tube no. 5), respectively. In case of *B. cereus* and *S. aureus* the growth of these micro-organisms were observed in the test tube no. 7 at a concentration 8 µg/ml, indicating that the MIC value of the methanolic bark extract was 16 µg/ml (test tube no. 6) (Table 5).

However, the antifungal activity, *A. niger, R. stolonifer, A. solani* and *F. oxisporum* the growth of these micro-organisms were observed in the test tube no. 7 at a concentration 8 µg/ml, indicating that the MIC value of the methanolic stem extract was 16 µg/ml (test tube no. 6).Further, in *A. flavus* the growth of these micro-organisms were observed in the test tube no. 6 at a concentration 16 µg/ml, signifying that the MIC value of the methanolic stem extract was 32 µg/ml (test tube no. 5) (Table 5).

DISCUSSION

In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide. It is aroused due to indiscriminate and repetitive use of antimicrobial drugs by inadequate disease treatment. To acquire drug resistance microbes have developed new enzyme system to cleave the drug and make it useless for control of infection. Hence, plant origin herbal medicines are considered as safe alternatives of synthetic drugs, which later on become an integral part of primary health care in many parts of world.Hence, plants, which possess strong antimicrobial potential against pathogens are considered as valuable source of medicinal compounds and show lesser side effects.

In the present investigation methanolic extracts of the different plant parts of *Tinospora cardifolia* was screened for their antimicrobial activities. Among the ten microbes tested (*E. coli, E. cloacae, B.cereus, S. aureus, P. aeruginosa, A. niger, A. flavus, A. solani, R. stolonifer* and *F. oxisporum*), extracts of the various plant parts showed antimicrobial activity against all the tested microorganisms.

The methanolic extracts of different plant part extract *Tinospora cardifolia* showed significant antimicrobial activity against multi-drug resistant clinically isolated microorganisms. Similar results showing that the alcoholic extract having the best antimicrobial activity was also reported by Preethi [24] *in Leucas aspera, Holarrhena antidysenterica.* Furthermore, Rajendran and Ramakrishanan [25] also observed that the Methanolic extract inhibits the growth of bacteria *P. aeruginosa, E.coli* and *S. aureus* than aqueous extract. Seyydnejad [26] also studied the effect of different alcoholic viz. ethanol and methanol for antimicrobial activity and observed that this difference in the activity between different alcoholic extract was due to the difference between extract compounds in these two extracts. All the above mentioned findings were in consonance of our present studies.

The antimicrobial analysis using the agar well diffusion method and MIC value has also been used by many other researchers Arora and Kaur,; Gurudeeban, ; Pavithra [27].

Table: 1- Inhibition zone diameters in methanolic extract of different plant parts of *Tinospora cardifolia* were measured by Agar well diffusion method (in mm).

Test Organisms		Inihibitation Zone Diameter (in mm)			
		Root	Stem	Leaf	Bark
Gram +ve Bacteria	*B. cereus*	9.0 ± 0.35	8.0 ± 0.13	10.8 ± 0.43	8.0 ± 0.16
	E. cloacae	9.4 ± 0.13	9.0 ± 0.39	14.5 ± 0.62	10.0 ± 0.30
	S. aureus	15.0 ± 0.32	11.0 ± 0.45	15.5 ± 0.14	12.6 ± 0.52
Gram -ve Bacteria	*E.coli*	8.8 ± 0.13	9.5 ± 0.82	14.0 ± 0.51	10.3 ± 0.45
	P.aeruginosa	12.0 ± 0.24	10.8 ± 0.39	11.0 ± 0.50	6.0 ± 0.79
Fungi	*A. niger*	14.6 ± 0.56	9.2 ± 0.35	15.0 ± 0.43	9.0 ± 0.19
	R. stolonifer	9.0 ± 0.39	8.7 ± 0.13	10.0 ± 0.39	6.2 ± 0.62
	A. flavus	9.8 ± 0.19	6.6 ± 0.36	9.3 ± 0.52	9.0 ± 0.13
	A. solani	9.6 ± 0.39	7.0 ± 0.32	10.0 ± 0.62	8.3 ± 0.89
	F. oxisporum	8.0 ± 0.29	7.8 ± 0.19	12.0 ± 0.66	6.0 ± 0.45

Values are mean inhibition zone (mm) ± S.D of three replicates

Table: 2- MIC of the root extract of *Tinospora cardifolia.*

Marked No. of test tubes	Nutrient Broth medium added (ml)	Diluted solution of Root extract (µg/ml)	Inoculums added (µl)	Growth observed against									
				Gram +ve Bacteria			Gram -ve Bacteria		Fungi				
				B. cereus	*E. cloacae*	*S. aureus*	*E. coli*	*P. Aeruginosa*	*A. niger*	*R. stolonifer*	*A. flavus*	*A. solani*	*F. oxisporum*
1	1	512	10	-	-	-	-	-	-	-	-	-	-
2	1	256	10	-	-	-	-	-	-	-	-	-	-
3	1	128	10	-	-	-	-	-	-	-	-	-	-
4	1	64	10	-	-	-	+	-	-	-	-	-	-
5	1	32	10	-	-	-	+	-	-	-	-	-	-
6	1	16	10	+	+	+	+	-	+	-	+	-	+
7	1	8	10	+	+	+	+	+	+	+	+	+	+
8	1	4	10	+	+	+	+	+	+	+	+	+	+
9	1	2	10	+	+	+	+	+	+	+	+	+	+
T_{MC}	1	512	10	-	-	-	-	-	-	-	-	-	-
T_{MI}	1	0	10	+	+	+	+	+	+	+	+	+	+
T_{M}	1	0	10	-	-	-	-	-	-	-	-	-	-

(+) = Indicates growth (-) = indicates no growth

Table: 3- MIC of the methanolic stem extract of *Tinospora cardifolia.*

Marked No. of test tubes	Nutrient Broth medium added (ml)	Diluted solution of Root extract (µg/ml)	Inoculums added (µl)	Growth observed against									
				Gram +ve Bacteria			Gram -ve Bacteria		Fungi				
				B. cereus	*E. cloacae*	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*R. stolonifer*	*A. flavus*	*A. solani*	*F. Oxisporum*
1	1	**512**	10	-	-	-	-	-	-	-	-	-	-
2	1	**256**	10	-	-	-	-	-	-	-	-	-	-
3	1	**128**	10	-	-	-	-	-	-	-	-	-	-
4	1	**64**	10	+	-	+	-	-	-	-	+	+	-
5	1	**32**	10	+	+	+	+	-	+	-	+	+	-
6	1	**16**	10	+	+	+	+	+	+	-	+	+	+
7	1	**8**	10	+	+	+	+	+	+	+	+	+	+
8	1	**4**	10	+	+	+	+	+	+	+	+	+	+
9	1	**2**	10	+	+	+	+	+	+	+	+	+	+
T_{MC}	1	**512**	10	-	-	-	-	-	-	-	-	-	-
T_{MI}	1	**0**	10	+	+	+	+	+	+	+	+	+	+
T_{M}	1	**0**	10	-	-	-	-	-	-	-	-	-	-

(+) = Indicates growth (-) = indicates no growth

Table: 4- MIC of the methanolic leaf extract of *Tinospora cardifolia.*

Marked No. of test tubes	Nutrient Broth medium added (ml)	Diluted solution of Root extract (µg/ml)	Inoculums added (µl)	Growth observed against									
				Gram +ve Bacteria			Gram -ve Bacteria		Fungi				
				B. cereus	*E. cloacae*	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*R. stolonifer*	*A. flavus*	*A. solani*	*F. Oxisporum*
1	1	**512**	10	-	-	-	-	-	-	-	-	-	-
2	1	**256**	10	-	-	-	-	-	-	-	-	-	-
3	1	**128**	10	-	-	-	-	-	-	-	-	-	-
4	1	**64**	10	-	-	-	-	-	-	-	-	-	-
5	1	**32**	10	-	-	-	-	-	-	-	-	-	-
6	1	**16**	10	-	+	-	+	-	-	-	+	-	-
7	1	**8**	10	+	+	+	+	+	+	+	+	+	+
8	1	**4**	10	+	+	+	+	+	+	+	+	+	+
9	1	**2**	10	+	+	+	+	+	+	+	+	+	+
T_{MC}	1	**512**	10	-	-	-	-	-	-	-	-	-	-
T_{MI}	1	**0**	10	+	+	+	+	+	+	+	+	+	+
T_{M}	1	**0**	10	-	-	-	-	-	-	-	-	-	-

(+) = Indicates growth (-) = indicates no growth

Table: 5- MIC of the methanolic bark extract of *Tinospora cardifolia.*

Marked No. of test tubes	Nutrient Broth medium added (ml)	Diluted solution of Root extract (µg/ml)	Inoculums added (µl)	Growth observed against									
				Gram +ve Bacteria			Gram -ve Bacteria		Fungi				
				B. cereus	*E. cloacae*	*S. aureus*	*E. coli*	*P. aeruginosa*	*A. niger*	*R. stolonifer*	*A. flavus*	*A. solani*	*F. oxisporum*
1	1	**512**	10	-	-	-	-	-	-	-	-	-	-
2	1	**256**	10	-	-	-	-	-	-	-	-	-	-
3	1	**128**	10	-	-	-	-	-	-	-	-	-	-
4	1	**64**	10	-	-	+	-	-	+	+	+	-	+
5	1	**32**	10	+	-	+	+	-	+	+	+	+	+
6	1	**16**	10	+	+	+	+	+	+	+	+	+	+
7	1	**8**	10	+	+	+	+	+	+	+	+	+	+
8	1	**4**	10	+	+	+	+	+	+	+	+	+	+
9	1	**2**	10	+	+	+	+	+	+	+	+	+	+
T_{MC}	1	**512**	10	-	-	-	-	-	-	-	-	-	-
T_{MI}	1	**0**	10	+	+	+	+	+	+	+	+	+	+
T_{M}	1	**0**	10	-	-	-	-	-	-	-	-	-	-

(+) = Indicates growth (-) = indicates no growth

CONCLUSION

In the continuation of global effort for the isolation of new and potent bioactive principles from plants, the present study has focused on the determination of minimum inhibitory concentration (MIC) of different *in vivo* plant parts of *Tinospora cardifolia* of Menispermaceae family, which may play an important role in the modern drug discovery program.

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