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Antifungal activity of Methanolic and Ethanolic Stem and Leaves extracts of Tinospora cordifolia

Garg Praveen[1]

Science Department, VITS College, Satna, (M.P.), India

Abstract: *The antifungal activity of the methanol and ethanol extracts of leaves and stem of Tinospora cordifolia was studied using agar well diffusion method against Aspergillus flavus and Candida albicans. In this study, solvent extracts was used in various concentrations. The results suggested that methanolic leaves and stem extracts has higher antifungal activity in comparision to ethanolic leaves and stem extracts against test fungal species in the form of inhibition zone. The present study suggested that different parts of Tinospora cordifolia used in medicinal purpose to cure various infectious disease.*

Key words: Antifungal activity, Infectious disease, Inhibition zone, Tinospora cordifolia.

Introduction

Plant-derived medicines have been part of traditional health care in most parts of the world for thousands for year [1]. In India, medical plants are widely used by all sections of people either directly as folk remedies or in different indigenous medicinal plants and their therapeutic values [2]. One of the plants known for having many medicinal use in traditional system of medicinal *Tinospora cordifolia* (Menispermeaceae). Considering the uses of *Tinospora cordifolia* in traditional system of medicine, we have proposed to work on this aspect to evaluate its antibacterial activities. This vast majorty of knowledge is commonly known to as the 'mother of all healing'. Ayurveda recommends that *Tinospora cordifolia* enhances normal resistance power of body, developed long life activity, it act as anti-stress medicine and an adaptogen [3, 4]. The complete plants contain varied bioactive compounds like antiulcer, hypolipidemic, [5] antipyretic, anticancer. In ayurvedic medicative system, it's used as most divine shrubs for its enormous properties like as medicinal drug, antiarthritis, antidiabetic, inhibitor, antiallergic, antileprotic, antiperiodic, hepatoprotective, antiprotozoal, antineoplastic and immunomodulatory activities [6, 7]. The *Tinospora cordifolia* root has been clinically used to treat jaundice, rheumatoid arthritis, and diabetes [8]. *Tinospora cordifolia* has widely used in ayurvedic, unani, and homoeopathy. The benefits of health observed may be because of the presence of the numerous phytochemicals such as terpenes, phenols, flavonoids, anthocyanins, alkaloids, and glycosides.

Material and methods

Plant material

Tinospora cordifolia were collected from the rural area of Chitrakoot (M.P.), in the month of April 2015. Stems and Leaves of this plant were collected for the current study and used it for further experiments.

Grinding of selected plant material:

The leaves and stems were taken and washed with running water slowly to separate soil and other contaminant particles. After this, again it cleaned with sterilized distilled water. The plant material (leaves and stem) were collected and dried at 37 deg C. The dried sample of plant was cut off into small pieces and grounded to powdered form in the mixer. These powdered samples of plant were kept on in an airtight container of glass for furthermore extraction process.

Extraction of plant materials:

Powdered and shed dried plant samples (100gm of each) were extracted with different solvents such as chloroform, ethanol, and methanol using maceration process [9]. Then, the extract was filtered and allowed to evaporate the solvent in a rotary evaporator at 40 deg C and both plant extracts were resuspended in respective solvents for further study.

Culture media and Microorganism

Taken a commercial PDA media (agar, dextrose, and potato starch) was dissolved in distilled water. The flask containing medium was tightly plugged with cotton and put into an autoclave for sterilization. The sterilization process is completed at 15 lbs /inch2 (121 deg C) for 15 minutes.

Table No.1:- Name of Pathogenic Fungus

S. No.	MTCC NO.	Microorganism Strains
1	MTCC – 277	*Aspergillus flavus*
2	MTCC – 227	*Candida albicans*
3	MTCC – 16888	*Aspergillus niger*
4	MTCC – 1663	*Alternaria alternate*

Agar well diffusion method

This method is used to determine the antifungal activity of all extracts prepared from the plant material of *Tinospora cordifolia* using the standard procedure [10]. In this method, taken a Potato dextrose agar plates with developed fungal culture and prepared wells with the help of sterile cork borer. There were 3 concentrations used of each extracted phytochemicals of the plant such as methanol and ethanol extracts (leaf and stem) of

Tinospora cordifolia, which are 25, 50 and 100 mg/ml. Different concentrations of plant extracts samples (25, 50, 100mg/ml) were added into wells with a sterile syringe and allowed for diffuses for 2 hours at room temperature. A similar process repeats with an antibiotic drug as a control sample. After inoculation with the tested organism on the agar surface, it is necessary to plant sample and antibiotics added into wells. The plates kept for incubation at 37 deg C for 24 hours and then examined clear zones of inhibition surrounded the wells with a particular concentration of the drug and plant extracts sample. A clear zone of inhibition on the plates indicates the antifungal activity of plant extracts.

Results and Discussion

Results of antifungal activity of the leaves and stem extracts of *Tinospora cordifolia* were measured in terms of inhibition. Antifungal activity of different solvent extracts of *Tinospora cordifolia* were obtained and evaluated against two fungal pathogens *A. flavus* and *C. albicans.* These fungal species also tested against standard drug fluconazole. Results of standard drug are concluded in table no. 2 (figure 2) which clearly shown the *C. albicans* is more effective than *A. flavus* in minimum concentration. The result of antifungal activity of different extracts of *Tinospora cordifolia* has given below in the table no.3 (figure no. 3,4).

Table No. 2: Antifungal activity of standard drug on different Anti microbial agents

S.N	Name of standard drug	Microbes	Zone of inhibition		
1	Fluconazole	*Aspergillus flavus*	**25µg/ml**	**50 µg/ml**	**100 µg/ml**
			15±0.09	18±0.17	20±0.01
		Candida albicans	**10 µg/ml**	**20 µg/ml**	**30 µg/ml**
			16±0.04	20±0.09	28±0.11

Table No. 3: Antifungal activity of *Tinospora cordifolia* extracts on different Microbes

S. No.	Name of microbes	Zone of inhibition		
		Leaves (methanolic extract)		
		25mg/ml	**50 mg/ml**	**100mg/ml**
1	*Aspergillus flavus*	11±0.12	14±0.15	20±0.11
2	*Candida albicans*	12±0.14	13±0.15	20±0.08
Stem (methanolic extract)				
1	*Aspergillus flavus*	10±0.11	12±0.05	13±0.13
2	*Candida albicans*	9±0.17	11±0.18	19±0.18

Leaves (ethanolic extract)				
1	*Aspergillus flavus*	12±0.12	13±0.12	18±0.11
2	*Candida albicans*	9±0.19	13±0.05	14±0.08
Stem (ethanolic extract)				
1	*Aspergillus flavus*	13±0.12	17±0.15	18±0.14
2	*Candida albicans*	8±0.14	10±0.15	11±0.08

Zone of inhibition (ZOI) values are reported as mean ± SD of three replicates.

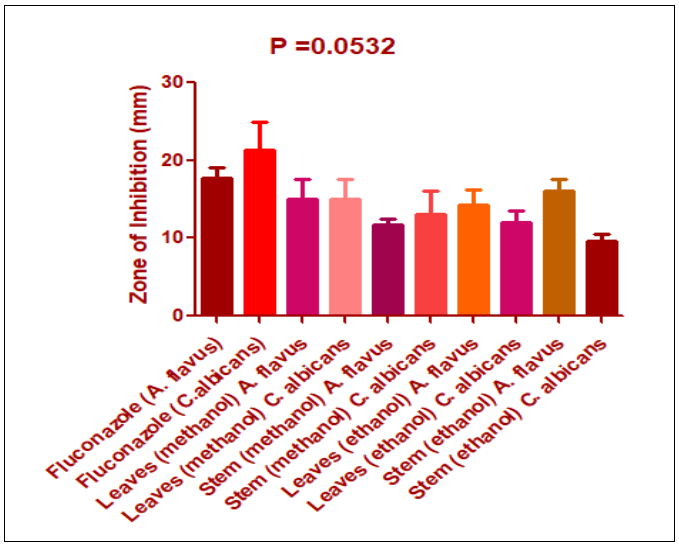

Figure 1: Antifungal activity of different solvent extract of *Tinospora cordifolia against Aspergillus flavus and Candida albicans*

One-way analysis of variance	
P value	0.0532
P value summary	Ns
Are means signif. different? (P < 0.05)	No
Number of groups	10
F	2.354
R squared	0.5144

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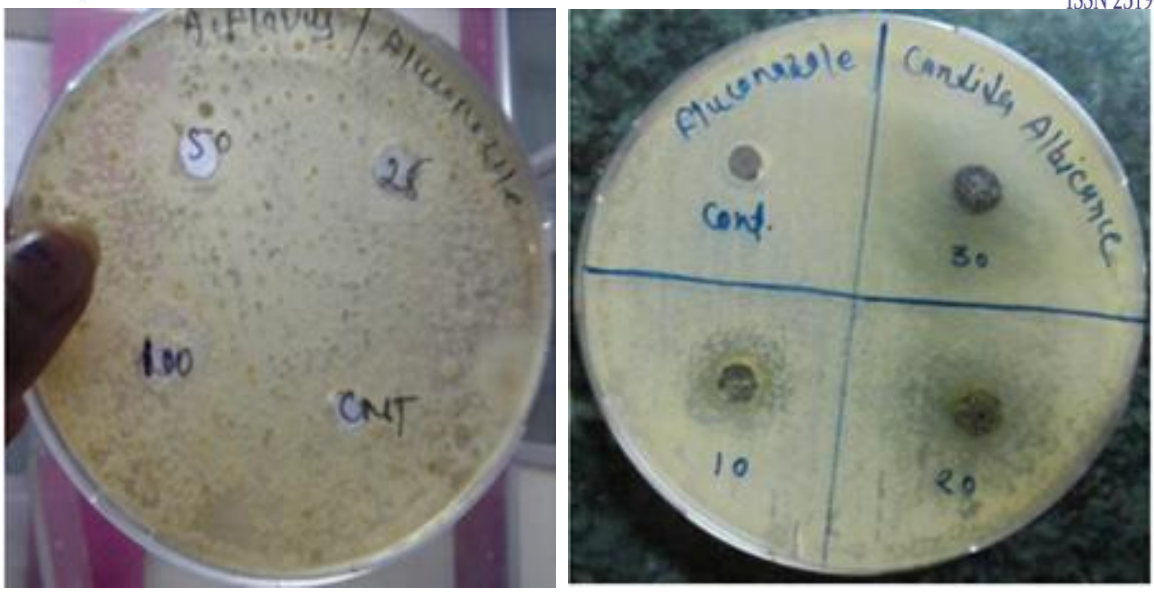

(A) (B)

Figure 2: (A) - Antifungal activity of standard drug fluconazole against *Aspergillus flavus* (B) - Antifungal activity of standard drug fluconazole against *Candida albicans*

(A) (B)

(C) (D)

Figure no. 3: Antifungal activity of (A & B) - Methanol leaf and stem extracts of *Tinospora cordifolia* against *Aspergillus flavus* (C & D) - Methanol leaf and stem extracts of *Tinospora cordifolia* against *Candida albicans*

(A) (B)

(C) (D)

Figure no. 4: Antifungal activity of (A & B) - Ethanol leaf and stem extracts of *Tinospora cordifolia* against *Aspergillus flavus* (C & D) - Ethanol leaf and stem extracts of *Tinospora cordifolia* against *Candida albicans*

According to table 3 (figure 3 and 4), Whereas methanolic leaves extracts of *Tinospora cordifolia* exhibited similar inhibition zone against *C. Albicans* and *A. flavus*, in which not so many differences, whereas methanolic stem extracts of *Tinospora cordifolia* exhibited maximum zone of inhibition against *C. Albicans* followed by *A. flavus*. Ethanolic leaf extracts of *Tinospora cordifolia* exhibited a highest zone of inhibition against *A. flavus* than *C. Albicans*, whereas ethanolic stem extracts of *Tinospora cordifolia* has shown maximum zone of inhibition against *A. flavus* than *C. Albicans*.

The highest zone of inhibition of plant extracts sample and standard solution (fluconazole) represents the high antifungal activity against both clinical fungi such as *A. Flavus* and *C. Albicans.* Acoording to table no. 2 (figure 2), *C. albicans* has shown maximal zone of inhibition in minimum concentration than *A. flavus* against standard drug (fluconazole). *Tinospora cordifolia* represents high antifungal activity in methanolic leaves extracts against both *A. flavus* and *C. albicans* followed by ethanolic extracts (leaves and stem) while methanolic stem extract has high antifungal activity against *C. albicans*.

The present study thus pointed out that *Tinospora cordifolia* plants are effective against fungal infections caused by *A. flavus and C. albicans.*

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