

# Antifungal activities of ethanol extraction of Gurju (*T. cordifolia*) steam

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## Abstract

*Tinospora cordifolia* is medicinal plant commonly known as Guduchi or Gurjo with small greenish flowers, having enormous medicinal value in all its parts such as leaves, stem, and also the root. The main objective of this research was designed to assess the antifungal activity of ethanol extraction of *T. cordifolia* stem of different concentrations by Agar well diffusion method in the microbiology laboratory of Padma Kanya Multiple Campus for the duration of 3 months. In this study, only the stem was selected which was grinded in powder form then extraction was done in ethanol. The extract was diluted in DMSO of different concentration of 2%, 3%, 4%, 5%, 6%, 7% and 8%. Preliminary qualitative phytochemical screening was carried out for the presence of tannins, flavonoids and alkaloids. From this study, the extraction of *T. cordifolia* stem showed the presence of tannins, flavonoids and alkaloids. From various concentrations of 2%, 3%, 4%, 5%, 6%, 7%, and 8% of *T. cordifolia*, antifungal activity (except for *Penicillium* spp.) was found to be highly effective in 6%, 7%, and 8% concentrations. This study concluded that the higher concentration of ethanol extraction of *T. cordifolia* was found to be effective for fungi. Therefore, for further studies, ethanol extraction for *T. cordifolia* is recommended to be used for antifungal activity.

**Keywords:** *Tinospora cordifolia*, Ethanol Extraction, Agar well diffusion, Fungi

## Introduction

*T. cordifolia* is widely recognized as Guduchi or Gurjo is a traditional medicinal plant belonging to the Menispermaceae family of moonseeds (Tiwari *et al.*, 2014). *Tinospora cordifolia* is a deciduous climbing shrub with small greenish flowers, having enormous medicinal value in all its parts such as leaves, stem, and also the root (Pandey *et al.*, 2012).

The scientific classification of this medicinal herb is given below;

## Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Ranunculales

Family: Menispermaceae

Genus: *Tinospora*

Species: *T. cordifolia*



Fig.1: *Tinospora cordifolia*

*T. cordifolia* contains 4.5%-11.2% ample protein, 15.9% fiber, 61.66% carbohydrates, 3.1% low fat, 0.845% potassium, 0.006% chromium, 0.28% iron and calcium 0.131% calcium. It contains nutritional value of 292.54 calories per 100 g (Khan, *et al.*, 2011). The studies of analysis of phytochemical of plant indicates the presence of a wide varieties of phyto-constituents which are lactones, alkaloids, glycosides, diterpenoid, sesquiterpenoids, hormones, aliphatic compounds, phenolics, and actively presence of ingredients like polysaccharides (Thatte *et al.*, 1991; Khan *et al.*, 2016).

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In South Asia, *T. cordifolia* is known as indigenous medicine which was used in various places in Nepal (Shrestha and Pandit, 2018). In Nepal, Residents of the Chitwan district used *T. cordifolia* for the treatment of jaundice, fever, cough, skin diseases, asthma, leprosy, uropathy, gonorrhea, splenopathy, immuno modulator and gout, (Maharjan *et al.*, 2012). In Parsa district, Tharu are still using *T. cordifolia* for the treatment of different diseases like jaundice, arthritis, diabetes and rheumatoid (Singh, 2020). *T. cordifolia* is known for antibacterial and antifungal agent and insecticide (Krishna *et al.*, 2009; Saha *et al.*, 2012). Antimicrobial activity of *T. cordifolia* are found in extraction of stem, root and leaf against pathogenic microorganisms (Panchabhai *et al.*, 2008). However, there is no much more research on antimicrobial activity of *T. cordifolia* against the fungi. Hence, the present study was aimed to assess the antimicrobial activity of *T. cordifolia* against the fungi.

## Methods and Materials

**Study design:** A cross-sectional study was done for the duration of 3 months. This study was designed to assess the antifungal activity of different concentrations of *T. cordifolia* stem in the microbiology laboratory of Padma Kanya Multiple Campus.

### Sample collection:

For the study, only stem of *T. cordifolia* was selected from the mature plants of *T. cordifolia* and then was collected aseptically in clean plastic bag and transported to the Laboratory of Padma Kanya Multiple Campus.

### Laboratory processing:

*T. cordifolia* stem was used for ethanol extraction. *T. cordifolia* stem powder was prepared and sieved to obtain uniform sized particles. For the extraction of *T. cordifolia* stem, 30gm of finely sieved powder was macerated with 100mL of 100% ethanol. Then the mixture was subjected to filtration with Whatman filter paper (No. 1) to obtain a clear filtrate. This filtrate was reduced at 80°C temperature in a water bath for 48 hrs to get a solid residue of *T. cordifolia* extract. Then, one gram of each extract (Ethanol extraction) was dissolved in 10 mL of 10% Dimethyl sulfoxide (DMSO) which was used for phytochemical screening and for antifungal study (Patil, *et al.*, 2017).

**Phytochemical screening:** Preliminary qualitative phytochemical screening was carried out with the following methods (Khandelwal, 2001).

**Test for Tannins:** In 0.5 mL of *T. cordifolia* extract solution, 1 mL of distilled water was mixed then 1 to 2 drops solution of ferric chloride was added and observed for green black or blue coloration.

**Test for Flavonoids:** A volume of 1.5 mL of 50 % methanol was added to 4 mL of the extracts. The solution and magnesium metal was added and warmed. Then, 5 to 6 drops of concentrated hydrochloric acid was added to the solution and observed for red coloration.

**Test for Alkaloids:** To 4 mL of extract filtrate, a drop of Mayer's reagent was added along the sides of the test tube.

### Preparation of different concentrations:

When one gram of each extract (Ethanol extraction) was dissolved in 10 mL of 10% Dimethyl sulfoxide (DMSO), 10% concentration of extract was obtained. Then, 1mL of this extract was transferred to a sterilized test tube and was labeled as 10% which was used as whole concentration samples. The remaining of the extract was again diluted further with DMSO to obtain seven different concentrations of 2%, 3%, 4%, 5%, 6%, 7%, and 8% (Agarwal *et al.*, 2010).

### Antifungal activity (Agar well diffusion method)

All tested fungi were available in the Laboratory of Padma Kanya Multiple Campus. All fungi were subcultured on PDA and colony morphology and staining (Lactophenol Cotton Blue staining method) was performed for confirmation. In this study, four fungi *Aspergillus niger*, *Penicillium* spp., *Alternaria* spp., and *Fusarium* were used for antifungal activity. For antifungal properties, 0.1 mL

fungal suspension of  $10^5$  CFU mL<sup>-1</sup> was uniformly spread on PDA plate to form lawn cultures. Then, a cork borer of 6 mm diameter was sterile and pressed above the inoculated agar plates for making wells in the plate. In each well, 70  $\mu$ l different concentrations of extraction along with the antibiotic (positive control) and DMSO (negative control) was introduced and then incubated aerobically for 3 days to 7 days at 30°C to study the zone of inhibition (Singh and Sastri 1981).

### Quality control in the laboratory

Quality of laboratory was maintained during laboratory work like equipment's was monitored continuously throughout the study period. The quality and sterility of each agar plate prepared was also maintained by incubating one plate of each batch in an incubator at 37°C for 24 hours.

### Data analysis

The data obtained from the experiment was entered in Microsoft Excel and data were presented in tables.

### Result

From ethanol extraction of *T. cordifolia* stem, phytochemical screening showed for the presence of alkaloids, flavonoids and tannins.

**Table 1: Phytochemical screening of Gurju (*T. cordifolia*) steam extraction**

S. no	Phytochemical	Gurju ( <i>T. cordifolia</i> ) steam extraction
1.	Alkaloid	+
2.	Flavonoids	+
3.	Tannins	+

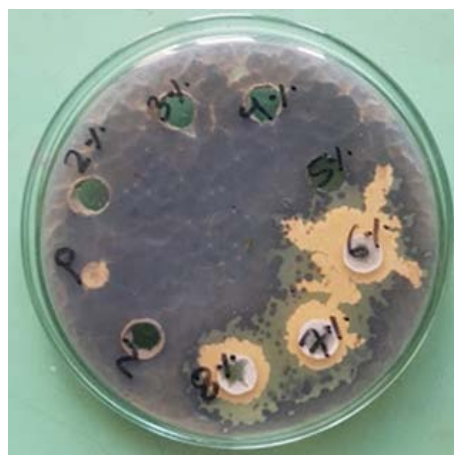
Among test fungi, *Aspergillus* spp. was inhibited by 6%, 7% and 8%. Similarly, *Alternaria* spp. was only inhibited by 8% concentration (high concentration). However, *Penicillium* spp. was not inhibited by any extraction.

**Table 2: Antifungal activity of different concentration of ethanol extraction of Gurju (*T. cordifolia*) steam**

S.no	Fungi	2%	3%	4%	5%	6%	7%	8%
1.	<i>Aspergillus</i> spp.	0	0	0	0	24	23	23
2.	<i>Penicillium</i> spp.	0	0	0	0	0	0	0
3.	<i>Alternaria</i> spp.	0	0	0	0	0	0	13



A



B

A. Ethanol extraction of powder Gurju plant stem

B. Antifungal activity of Ethanol extraction of Stem of *T. cordifolia* (P=Positive control and N= negative control)

## Discussion

In this study, ethanol extraction was extracted from stem of *T. cordifolia* plant. From this extraction, alkaloids, flavonoids and tannins were screened as phytochemical. Similar studies were reported by Krishna *et al.*, (2009) & Saha and Ghosh, (2012) in which alkaloids, flavonoids and tannins were found as phytochemical. In this study, only stem was used for ethanol extraction for the study of antimicrobial activity. However, Panchabhai *et al.*, (2008) reported antimicrobial features in different parts of *T. cordifolia* plant like in root, stem, and leaf extracts.

In this study, only antifungal activity was studied in the ethanol extraction of the *T. cordifolia* stem powder. However, Krishna *et al.*, (2009) & Saha and Ghosh, (2012) reported antifungal activity as well as antibacterial activity. Among test fungi, *Penicillium* spp. was not inhibited by ethanol extraction of stem of *T. cordifolia* plant. On the other hand, *Aspergillus* spp. was inhibited by 6%, 7% and 8% of concentration. However, *Alternaria* spp. was only inhibited by 8% concentration of ethanol extraction of *T. cordifolia*. The highest zone of inhibition was shown against *Aspergillus* spp. by 6% concentration which was 24mm. Similarly, study carried by Patil *et al.*, (2017), ethanolic extract showed growth inhibitory against *Candida albicans*, *Aspergillus niger*, *Aspergillus fumigates* and *Microsporium gypseum*.

During pandemic of COVID-19, *T. cordifolia* plant was widely used for the treatment of viral diseases. Some studies suggested for herbal medicine for reducing the severity as well as to prevent COVID-19 (Chan *et al.*, 2019; Vellingiri *et al.*, 2020). In pandemic time of COVID-19, China and India used many herbal medicine along modern medicine for enhancement of the immunity of patients (Shankar, *et al.*, 2020; Ni *et al.*, 2020).

## Conclusion

Ethanol extraction of *T. cordifolia* showed varying antimicrobial efficacy against different fungi. Among different concentration, 6%, 7% and 8% concentration of ethanol extracts were found to be highly effective for test fungi due to the presence of alkaloids, flavonoids and tannins.

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