

Assessment of Antimicrobial Potential of Acetone Extract of *Tinospora Cordifolia* Leaves against Pathogenic Microorganisms

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ABSTRACT:

Background: *Tinospora cordifolia* is a perennial shrubby vine that belongs to the Menispermaceae family. It is a plant with a lot of different phytochemical compounds that is used in traditional medicine. To evaluate the antimicrobial efficiency of *Tinospora cordifolia* leaf extract against *Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Candida albicans*, and *Candida auris*. **Materials and methods:** The agar well diffusion test was utilized to evaluate the antimicrobial efficacy of the acetone extractions from the *Tinospora cordifolia* leaf. The study utilized four distinct concentrations of the tested agents. **Result:** The acetone extracts were effective against the pathogens that were chosen of the plant extracts tested; *T. cordifolia* leaf extracts had the biggest zone of inhibition, which was 24.0 mm against *Proteus vulgaris* at a concentration of 100 mg/ml. **Conclusion:** The acetone extract of plants contained various phytochemicals suitable for pharmaceutical applications. This study determined that the *Tinospora cordifolia* plant possesses antimicrobial properties.

Keywords: Menispermaceae, antimicrobial activity, *Tinospora cordifolia*, pharmaceutical products, phytochemicals, pathogens.

I. INTRODUCTION

• The best place to obtain a large variety of medications is from plants that are used for medical purposes. Traditional medical practices in poor nations often involve the use of plants that have therapeutic properties. An extensive number of medicinal plants have been utilized for the treatment of a wide variety of diseases due to the fact that they possess antibacterial and antifungal properties.[1]

- These natural products are the source of the majority of pharmaceutically active chemicals. When it comes to natural goods, they are classified into many groups according to the location in the plant body from which they originate. These categories include a wide variety of compounds, such as alkaloids, polyphenols, polyketides, terpenoids, steroids, and saponins.[2]
- *Tinospora cordifolia*, which is also known as giloy, belongs to the family Menispermaceae. It is a significant medicinal plant that is utilized in ayurvedic medicine and possesses several curative capabilities, such as those for jaundice, rheumatism, urinary issues, skin illnesses, diabetes, anemia, inflammation, allergies, and a variety of other conditions.[3]
- *Tinospora cordifolia* can thrive in a wide variety of soil types, including those below. There is a climbing plant known as *Tinospora cordifolia*[4]. The leaves of this plant are simple, alternating, and long-petioled. Additionally, they are widely ovate, nerved, and deeply cordate at the base. Flowers blossom during the summer, and fruits are cultivated during the winter months. There are phytochemicals in these plants that are beneficial to your health and have the potential to influence your body in a physiological manner. A few examples of these components include phenolics, tannins, flavonoids, alkaloids, steroids, terpenoids, and any number of other substances.[5]
- Many different plant genetic resources have been investigated to determine whether or not they contain bioactive chemicals that possess antibacterial characteristics. As a result, antimicrobial activity can be exploited in the management of infectious disorders that are

caused by resistant microorganisms. These pathogens include *Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Candida albicans*, and *Candida auris*. Antimicrobial activity either kills bacteria or prevents them from developing.[6]



Figure 1: *Tinospora cordifolia* leaves.

II. MATERIALS AND METHODS

Collecting and authenticating plant materials:

The sample of *Tinospora cordifolia* leaves was collected from Kottur village, Krishnagiri, India, in November and authenticated by the Siddha medicinal plants garden, Mettur Dam, Salem, Tamil Nadu, India.

Preparation of plant materials:

T. cordifolia leaves were picked and washed under running water to get rid of dirt and then dried in the shade at room temperature for 15 to 20 days.

The plant material was cut into small pieces, ground into a fine powder in an electronic grinder, and put in airtight aluminum foil for later use. It was then stored at room temperature.

Preparation of plant extraction:

The extract was made by soaking the plant in acetone. We took 50 grams of powdered leaves of *Tinospora cordifolia*, which were soaked in 500 ml of acetone by using the maceration method with a magnetic stirrer for 12-24 hours.

Using a muslin cloth to filter the extracts. The acetone extracts were kept at 4°C until they were needed again.



Figure 2: maceration method of *Tinospora cordifolia* leaf

Phytochemical screening:

Different tests were done to make sure that *T. cordifolia* leaf extract contained phytochemicals like alkaloids, carbohydrates, terpenoids, steroids, saponins, tannins, amino acids, flavonoids, glycosides, and proteins. We did phytochemical analysis using the following standard methods.

Detection of Alkaloids[7]:

- Wagner test: A sample of plant extract with hydrochloric acid at a concentration of 1.5% volume/volume. Following this, a few drops of Wagner's reagent, which is a solution of iodine potassium iodide, are added to the test tube. It produced deposits that were a reddish-brown color, which is evidence that alkaloids are present.
- Mayer's test: A sample of plant extracts containing 2 ml was placed in a test tube, and then two to three drops of Mayer's reagent, which is a solution of potassium mercuric iodine, were added to the sample. When the sample was analyzed, a dingy white precipitate was produced if it contained alkaloids.

Detection of Proteins and Amino Acids:

- Bring a sample of plant extracts to a boil for one to two minutes after combining it with two drops of a freshly produced solution of ninhydrin with a concentration of 0.2%. After that, allow it to cool off. The presence of amino acids, proteins, and peptides is indicated by the color blue for the substance.

Detection of Saponins:

- The sample of alcohol, which was 1 ml in volume, was combined with 2 ml of distilled water. For a period of fifteen minutes, this solution was shaken inside of a graduated cylinder. It is possible that the extracts will produce a foam layer that is 1 cm thick if they include saponins.

Detection of Glycosides[8]:

- 1 ml of concentrated hydrochloric acid was added to the test solution, and it was allowed to settle for two minutes. The presence of glycosides was demonstrated by the formation of a precipitate that was purple in hue.

Detection of Tannins[9]:

- Following the addition of a few drops of ferric chloride at a concentration of 0.1%, the hue altered to a brownish-green or blackish-blue in appearance. Clearly, tannins are present, as evidenced by the hue.

Detection of Phenols:

- It was decided to combine 1 ml of extracts with a few drops of ferric chloride solution. There is evidence of the presence of phenols due to the bluish-black tint.

Detection of Carbohydrates[10]:

- It is necessary to add 1 ml of extract that has been heated to 1 ml of Fehling's A and 1 ml of Fehling's B. The presence of a precipitate that is brick red in color indicates the presence of carbohydrates.

Detection of Steroids[11]:

- Salkowski test involves adding a few drops of strong sulfuric acid to 1 ml of *Tinospora cordifolia* leaf extract in order to produce a reddish-colored solution. Steroids are present in the body.

Detection of Flavonoids:

- A few drops of yellow lead acetate solution were added to 1 ml of extract in order to treat it. Flavonoids are present, as shown by the coloration.

Detection of Terpenoids:

- In order to construct a layer, the extract was carefully applied together with 2 ml of chloroform and 3 ml of concentrated hydrosulfite. It is possible to determine whether or not terpenoids were present by observing the creation of a thin, reddish-brown hue.

Antimicrobial activity of *Tinospora cordifolia*[12, 13]:

Test microorganisms:

The investigation utilized a total of six bacterial strains. The bacterial and fungal cultures

were sourced from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The bacteria that were used are *Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Proteus vulgaris*. The fungi employed were *Candida albicans* and *Candida auris*.

Test for antibacterial properties:

Getting ready for inoculum:

We maintained stock cultures at 4°C on nutrient agar slopes. A loopful of cells from the stock cultures was transferred to a test tube containing Muller-Hinton broth (MHB) for bacteria in order to create active cultures for the experiment. After that, the test tube was incubated for 24 hours at 37°C without being shaken. The cultures were combined with new Muller-Hinton broth to achieve optical densities of 2.0×10^6 colony-forming units (CFU/ml) for bacteria (Smith & Doe 2018).

i. Making sterile swabs:

Cotton wool swabs were created and sterilized on plastic or wooden applicators using dry heat (just for the wooden swabs) or autoclaving. The swabs were sterilized by placing them in culture tubes, sheets, tins, and other containers.

ii. Sterilization of forceps:

You can clean forceps by dipping them in alcohol and burning the alcohol off.

Antibacterial assay with the agar well diffusion method:

For the purpose of determining the antibacterial activity, the well diffusion techniques were utilized. In order to evaluate the antibacterial activity in vitro, we utilized Muller-Hinton Agar (MHA), which was purchased from Himedia in Mumbai. A total of 15 ml of melted media was put into sterile petri plates in order to create the MHA plates. After allowing the plates to solidify for five minutes, an inoculum suspension containing 0.1% was swabbed onto them in an even manner. After five minutes, the inoculum could be allowed to dry. Before adding 20 µl of the test drug at various concentrations, the wells were cut and then refilled with the medication. Following that, the plates were stored at 37°C for a period of 24 hours. The diameter of the inhibition zone that surrounded the well was measured in order to determine whether or not the antibacterial activity was present (NCCLS, 1993). A disc containing

chloramphenicol was utilized by us as a positive control.

Antifungal activity:

Methods of well diffusion plates on agar were utilized in order to establish the level of antifungal activity. To determine whether or not the extract possessed antifungal properties, the fractions of various concentrations of the extract were dissolved in water. Each petri dish measuring 15 cm was filled with 20 ml of Sabouraud dextrose agar. *Candida albicans* and *Candida auris* were grown in Sabouraud dextrose broth for a period of 48 hours at a temperature of 27°C in order to achieve an optical density (OD) of 0.1% at 600 nm; the growth was diluted with Sabouraud dextrose. After the wells were cut, sample concentrations of the test substance were placed on the wells at

varied concentrations. These concentrations were 25, 50, 75, and 100 µl of each spice sample, with each sample containing 1 mg/ml. In order to serve as a positive control, we utilized one hundred units of fluconazole that we purchased from a nearby drugstore. The incubation period lasted for 48 hours at a temperature of 27°C.

III. RESULTS AND DISCUSSIONS
Phytochemical screening of *Tinospora cordifolia* leaf extract using acetone as a solvent:

For the purpose of determining whether or not the acetone extract of *Tinospora cordifolia* included any phytochemicals, a number of assays were carried out. It [Table 1] provides a summary of the whole test that was carried out as well as the outcomes of the test.

Phytochemicals	Results
Alkaloids	+
Flavonoids	+
Saponins	-
Carbohydrates	+
Glycosides	+
Tannins	+
Terpenoids	+
Proteins	+
Amino acids	+
Phenols	+
Steroids	-

Antimicrobial activity of *Tinospora cordifolia* leaf extract using acetone as a solvent:

The antibacterial activity of the leaf extract of *Tinospora cordifolia* was evaluated against six different kinds of bacteria. These bacteria were *Bacillus subtilis*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Proteus vulgaris*,

Candida albicans, and *Candida auris*. The bacterial and fungal strains that were produced were cultured in petri plates that had already been prepared. When testing the antibacterial activity of plant extract, several quantities of the extract were attempted.

S. NO	MICRO ORGANISMS	ZONE OF INHIBITION (MM)				
		25µL/ML	50µg/ML	75µg/ML	100µg/ML	+ ^{ve} control
Gram-positive bacteria						
1	<i>Bacillus subtilis</i>	11	14	17	20	23
2	<i>Enterococcus faecalis</i>	13	15	19	21	24
Gram-negative bacteria						
3	<i>Klebsiella pneumoniae</i>	12	16	18	20	23
4	<i>Proteus vulgaris</i>	14	16	20	24	26
Fungi						

5	Candida albicans	8	12	13	15	20
6	Candida auris	9	11	12	14	24

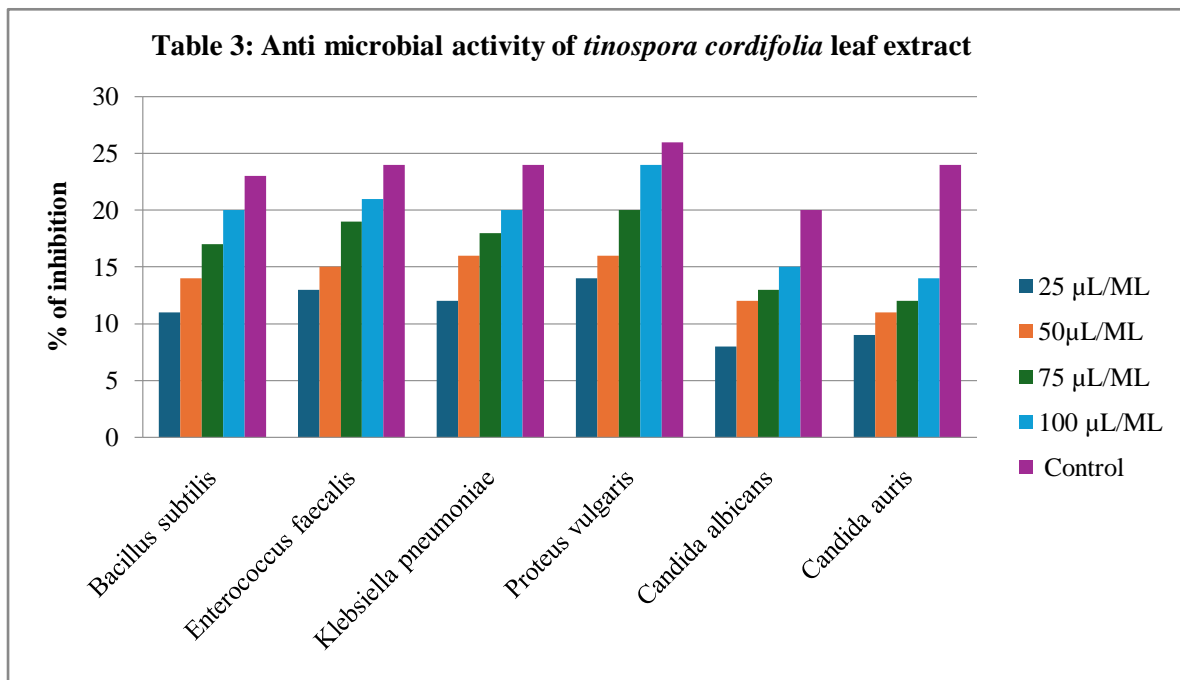


Figure 3: Inhibition of growth of Bacillus subtilis by acetone extract of *T. cordifolia* leaf

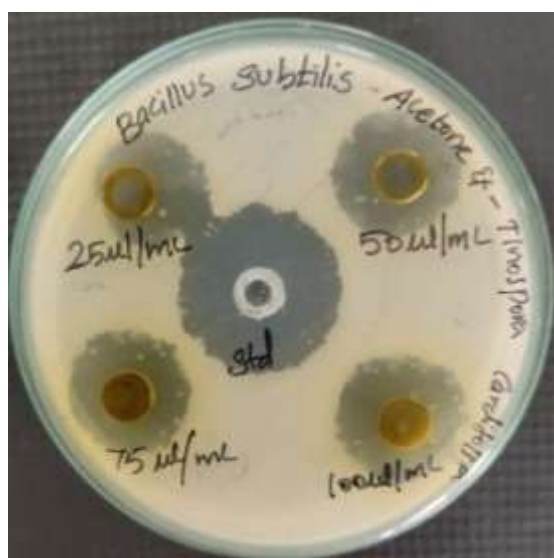


Figure 4: Inhibition of growth of Enterococcus faecalis by acetone extract of *T. cordifolia* leaf.

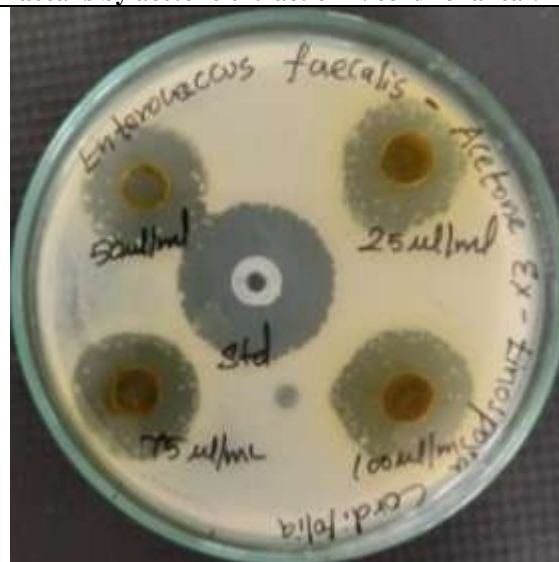


Figure 5: Inhibition of growth of Klebsiella pneumoniae by acetone extract of T. cordifolia leaf.



Figure 6: Inhibition of growth of Proteus vulgaris by acetone extract of T. cordifolia leaf.

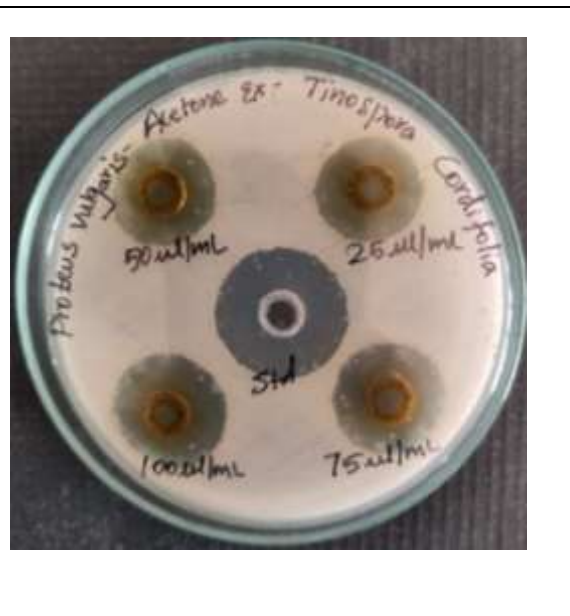


Figure 7: Inhibition of growth of Candida Albicans by acetone extract of T. cordifolia leaf.

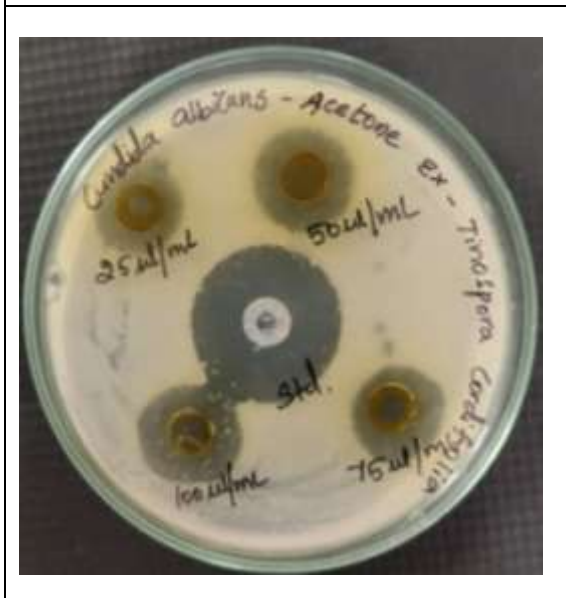
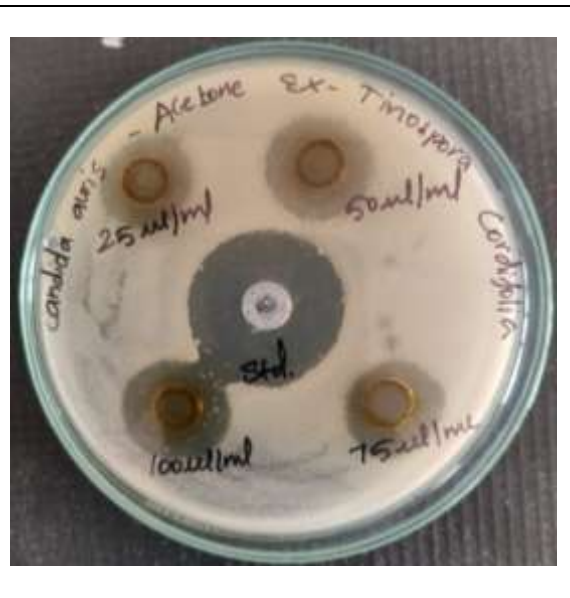


Figure 8: Inhibition of growth of Candida auris by acetone extract of T. cordifolia leaf.



IV. CONCLUSION

In the present study, the phytochemical screening for the leaf of Tinospora cordifolia showed the presence of active components like steroids, flavonoids, glycosides, tannins, alkaloids,

etc. The acetone extract of Tinospora cordifolia leaf possesses antimicrobial activity against Bacillus subtilis, Enterococcus faecalis, Klebsiella pneumoniae, Proteus vulgaris, Candida albicans, and Candida auris.

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Conflict of Interest:

The author(s) declares no conflict of interest.

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