



# The Antibacterial Effect of *Tinospora Cordifolia* (Guduchi) and Its Role in Combating Antimicrobial Resistance

## *Tinospora Cordifolia*'nın (Guduchi) Antibakteriyel Etkisi ve Antimikrobiyal Dirençle Mücadeledeki Rolü

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### ABSTRACT

**Objective:** *Tinospora cordifolia*, often known as "Guduchi," has a variety of medicinal characteristics, including those that are anti-diabetic, anti-inflammatory, anti-periodic, anti-arthritic, anti-oxidant, anti-allergic, and anti-stress. Drug development projects face significant obstacles because of the rapid rise in antimicrobial drug resistance in the world healthcare system and the introduction of multidrug-resistant (MDR) strains. In order to combat the MDR infections isolated from patients' bodies, the current study was conducted to evaluate and investigate the antibacterial capabilities of *Tinospora cordifolia*.

**Methods:** Using the agar well diffusion method, *Tinospora cordifolia* extracts (aqueous, chloroform, and ethanol) were tested for their antibacterial effectiveness against 30 MDR pathogens, and the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide assay was used to determine the minimal inhibitory concentration. To determine whether there were any medicinally useful and significant bioactive ingredients in the plant extract, phytochemical analysis was performed.

**Results:** In comparison to the aqueous extract which had little effect, the antibacterial activity of ethanol and chloroform extracts exhibited a potential antimicrobial effect against most of the tested bacterial isolates. Phytochemical analysis of *Tinospora cordifolia* extract showed alkaloids, carbohydrates, phytosterols, saponins, tannins, proteins, flavonoids, and terpenoids.

**Conclusions:** Our findings show that *Tinospora cordifolia* plays a potential role in combating the antimicrobial resistance of clinical isolates.

**Keywords:** Antimicrobial, MIC, multidrug resistant, plant extract, *Tinospora*

### ÖZ

**Amaç:** Genellikle "Guduchi" olarak bilinen *Tinospora cordifolia*, anti-diyabetik, anti-enflamatuvar, anti-periodik, anti-artritik, anti-oksidan, anti-alerjik ve anti-stres dahil olmak üzere çeşitli medikal özelliklere sahiptir. İlaç geliştirme projeleri, dünya sağlık sisteminde antimikrobiyal ilaç direncindeki hızlı artış ve çoklu ilaca dirençli (MDR) suşların piyasaya sürülmesi nedeniyle önemli engellerle karşılaşmaktadır. Bu çalışma hastaların vücutlarından izole edilen MDR enfeksiyonlarla mücadele etmek amacıyla, *Tinospora cordifolia*'nın antibakteriyel yeteneklerini değerlendirmek ve araştırmak için yapılmıştır.

**Yöntemler:** Agar kuyucuk difüzyon yöntemi kullanılarak, *Tinospora cordifolia* ekstraktlarının (sulu, kloroform ve etanol) 30 MDR patojene karşı antibakteriyel etkinlikleri test edildi ve minimum inhibitör konsantrasyonu belirlemek için 3-(4,5-dimetil-tiyazol-2-il)-2,5-difenil-tetrazolyum bromür testi kullanıldı. Bitki ekstraktında tıbbi olarak yararlı ve önemli biyoaktif içeriklerin olup olmadığını belirlemek için fitokimyasal analizler yapıldı.

**Bulgular:** Çok az etkiye sahip olan sulu ekstraktla karşılaştırıldığında, etanol ve kloroform ekstraktlarının antibakteriyel aktivitesi, test edilen bakteriyel izolatların çoğuna karşı potansiyel bir antimikrobiyal etki sergiledi. *Tinospora cordifolia* ekstraktının fitokimyasal analizi, alkaloidler, karbohidratlar, fitosteroller, saponinler, tanenler, proteinler, flavonoidler ve terpenoidler gösterdi.

**Sonuçlar:** Bulgularımız, *Tinospora cordifolia*'nın klinik izolatların antimikrobiyal direnciyle mücadelede potansiyel bir rol oynadığını göstermektedir.

**Anahtar kelimeler:** Antimikrobiyal, MIC, çoklu ilaca dirençli, bitki özü, *Tinospora*

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## INTRODUCTION

*Tinospora cordifolia*, commonly referred to as "Guduchi," has many medicinal properties such as anti-diabetic, anti-inflammatory, anti-periodic, anti-arthritis, anti-oxidant, anti-allergic, and anti-stress<sup>1</sup>. All parts of this medicinal plant, like the leaves, stem, and roots, show therapeutic and medicinal activities. The antibacterial properties of *Tinospora cordifolia* extracts have been tested and proven effective against bacterial agents like *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus vulgaris*, *Salmonella typhi*, *Shigella flexneri*, *Salmonella paratyphi*, *Salmonella typhimurium*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterobacter aerogenes* and *Serratia marcescens*<sup>1</sup>. It contains phytochemical compounds like terpenoids, alkaloids, ligands and steroids with antimicrobial effect against microbial strains<sup>2</sup>. *Tinospora cordifolia* is an important Indian drug that can be used as a potent agent in dealing with the increasing antimicrobial resistance today. Constituents from *Tinospora cordifolia* may be a potential source of newer therapeutic strategies for infectious diseases<sup>3</sup>. The World Health Organization reports that 80% of the current traditional medicines involving the use of plant extracts have provided a strong base for the general healthcare and alleviation of common ailments of the people<sup>4</sup>.

Rapid surges in antimicrobial drug resistance in global healthcare and emergence of multidrug-resistant (MDR) strains impose major hurdles in the progression of drug discovery programmes<sup>5</sup>. Newer antibiotic resistance mechanisms exhibited by the microbial community contribute to the inefficacy of the available drugs, thereby prolonging the medical illnesses and escalating the expenditures<sup>6,7</sup>. Medicinal plants like *Tinospora cordifolia* have shown promising effect as one of the important antimicrobial and disinfectant agents in the medical field with their bactericidal activity against Gram-positive and Gram-negative bacteria as well as antifungal and antiviral effect<sup>8-10</sup>.

*Tinospora cordifolia* exhibits potential antibacterial activity against the bacterial agents causing urinary tract infections, which could be used for treatment and proves promising against the MDR bacteria of clinical origin<sup>11,12</sup>. The presence of alkaloids, carbohydrates, and flavonoids makes this plant an effective alternative for clinical isolates with *in vitro* antimicrobial activity<sup>13,14</sup>. Plant-based therapeutic alternatives could be a useful way to tackle the emerging drug resistance in global healthcare facilities<sup>15</sup>. Very little exploratory work has been reported from Indian studies on biological and

therapeutic utility of *Tinospora cordifolia*. The creation of contemporary antimicrobial medications using the bioactive ingredients of *Tinospora cordifolia* to combat MDR organisms would be made possible by strengthening newer avenues such as drug research programmes. Utilizing phytochemicals from established plant sources such as *Tinospora cordifolia* can help reverse antibiotic resistance. Antibiotic resistance reversal is possible with the application of phytochemicals from traditional plant sources like *Tinospora cordifolia*. The research, clinical approval and use of these phytochemicals to combat MDR pathogens and related clinical issues will be accelerated by such plant-based investigations. Hence, the present study was undertaken to assess and explore the antibacterial properties of *Tinospora cordifolia* in combating antimicrobial resistance by the MDR pathogens isolated from the patients.

## MATERIALS and METHODS

The present prospective study was carried out in the Department of Microbiology and 30 MDR isolate like methicillin-resistant *Staphylococcus aureus* (MRSA) five isolates, two isolates of vancomycin resistant *Enterococci* (VRE) and eight extended spectrum beta lactam (ESBL) producers isolates which include *E. coli*, *K. pneumoniae* and *P. aeruginosa* along with six ATCC strains were included in the study.

The study was conducted after Sri Venkateshwarra Medical College Hospital and Research Centre Institutional Ethics Committee clearance (No. 03/SVMCH/IEC- Cert/June22, date: 19.07.2022) for two months from August 2022 to September 2022. Six ATCC strains and eight ESBL isolates were used in the study.

### Study Protocol

#### I. Collection of *Tinospora Cordifolia*

Fresh and healthy plant stems with leaves were collected from several locations, including the Pondicherry Botanical Garden, neighboring nurseries, and residential areas. Details like the name of the plant, its source, and date of collection were recorded (Figure 1).

#### II. Preparation of the Extracts<sup>16</sup>

a. Extracts were prepared based on Soxhlet method. The stem part of the plant (*Tinospora cordifolia*) was used in this experiment. The solvents used for the extraction were aqueous (water), chloroform, and ethanol.

b. The extraction was performed using succinate apparatus with standard protocol<sup>16</sup>. After thorough

cleaning and shade drying, the stems were made into fine powder using a pulverizer machine.

c. For the ethanol, chloroform and water solvents, the powder weights were 71.84 gm, 90.90 gm and 65 gm, respectively. The stem powders were placed in a porous bag, which was then placed in the chamber of the Soxhlet apparatus (Figure 2).

d. The extracting solvent (ethanol, chloroform, and water) in the flask was heated and its vapors condensed in the condenser. The condensed extract dripped into the porous bag containing crude extract and was extracted subsequently.

e. The liquid inside the siphon chamber enters the flask when the liquid level in the chamber reaches the top of the siphon tube.

f. This process is carried out continuously until an evaporated drop of solvent from siphon tube leaves no residue. Simultaneously, all three extracts (aqueous, chloroform and ethanol) were obtained by the Soxhlet apparatus with the same process. The final yield of 4.85% w/w, 2.004% w/w and 6.769% w/w was obtained for ethanol, chloroform and water solvents respectively.

### III. Test Organism, Inoculum Preparation and Antimicrobial Bioassay

a. The antibacterial effect of the extract of *Tinospora cordifolia* was tested on ATCC strains of Gram-positive and Gram-negative bacteria and on MDR bacterial strains.

b. The ATCC strains include *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *E. coli* ATCC 35218, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212. The strains were procured from the HiMedia Pvt. Ltd., India and are maintained as quality control bacterial strains in the Bacteriology section.

c. A total of 30 MDR organisms like MRSA (5), VRE (2) and ESBL producers (8) isolated for routine culture



Figure 1a, b. Plants of *Tinospora cordifolia*.

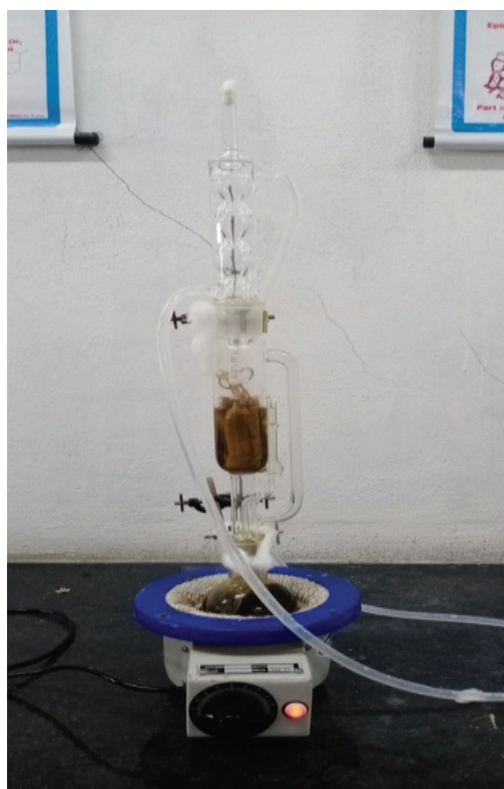


Figure 2. Soxhlet apparatus for the plant extraction.

sensitivity procedure in the bacteriology section were included in the study.

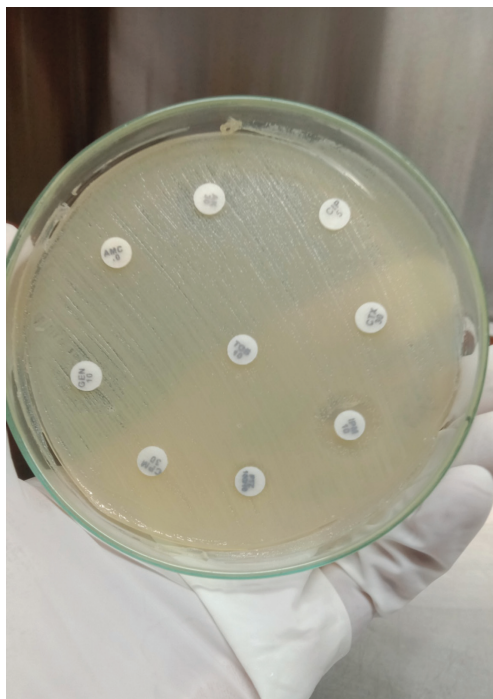
d. Antimicrobial susceptibility testing for the isolates was performed by the agar diffusion method according to the CLSI guidelines 2022 (Figure 3)<sup>17</sup>.

e. The antibacterial assay for aqueous, chloroform and ethanol extracts of *Tinospora cordifolia* were performed on Mueller Hinton Agar plate. The turbidity of the broth inoculum was compared with 0.5 McFarland Standards (HiMedia Pvt Ltd).

f. The agar well diffusion method was followed for the antibacterial assay. Using a sterile puncher, wells were made on the plate and 30  $\mu$ L of the extract was added to the wells, which were lawn cultured with the test and control organisms.

g. The quality control for the plant extracts was justified with available standard antibiotic i.e. ampicillin (30  $\mu$ g/disc) as positive control and for the negative control only solvent without the extract was added to the well.

h. These plates were kept at 4 °C for 2 hours (h) for the better diffusion of the test samples followed by 24 h of incubation at 37 °C.



**Figure 3.** MDR-antibiotic susceptibility testing.  
MDR: Multidrug-resistant

i. A clear and distinct zone of inhibition around the well was measured and interpreted as sensitive or resistant.

#### IV. Determination of Minimum Inhibitory Concentration (MIC)<sup>18</sup>

a. The MIC was determined by the broth microdilution for the 10 isolates that showed the zones of inhibition in the well diffusion method. The method was based on the reference method M38-P recommended by the National Committee for Clinical Laboratory Standards.

b. All solvent extracts (ethanol, chloroform, and water) were serially diluted using dimethyl sulfoxide (DMSO), the concentration ranging from 60  $\mu$ g/mL (stock) to dilution factor 1/320 (0.18  $\mu$ g/mL) in a 96 well plate. The inoculum of each isolate was prepared, and the suspensions were adjusted to 10<sup>6</sup> CFU/mL.

c. Each well of the microplate included 40  $\mu$ L of growth medium (Mueller Hinton broth MHB), 10  $\mu$ L of inoculum and 50  $\mu$ L of diluted extracts. Ampicillin and DMSO were used as positive and negative controls respectively.

d. Plates were then incubated at 37 °C for 24 h. Following incubation, 40  $\mu$ L of 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) at a final concentration of 0.5 mg/mL freshly prepared in water was added to each well and incubated overnight. MTT assay is the colorimetric agent. The yellow compound MTT is reduced by mitochondrial dehydrogenases to the water-insoluble blue compound formazan, depending on the viability of cells.

e. The change to red color showed that the bacteria are biologically active. The MIC was taken to the well where no color change in MTT was observed. To ensure the accuracy of the findings, the experiments were conducted three times.

#### V. Phytochemical Screening of Plant Extract (*Tinospora Cordifolia*)<sup>19-22</sup>

Preliminary phytochemical screening for the extract of *Tinospora cordifolia* was carried out at Sri Venkateshwara College of Pharmacy, Ariyur, Puducherry at Pharmacognosy Department. The plant extracts were screened for bioactive components like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, mucilage, flavonoids, terpenoids and fixed oil.

#### Statistical Analysis

Data were entered in a Microsoft Excel sheet, and analysis was done using IBM SPSS for Windows 22.0 for

the interpretation of results. Descriptive statistics were performed for the variables, and results were represented in the form of figures and tables for the current study.

## RESULTS

In the present study, the antimicrobial activity of three extracts (aqueous, chloroform and ethanol) of *Tinospora cordifolia* was tested against 30 MDR pathogens and their potential activity was qualitatively assessed by the presence or absence of inhibition zones. Positive control and negative tests were satisfactory. The sensitive strains were further tested for MIC values by MTT assay.

### Antibacterial Activity of *Tinospora Cordifolia*

The antimicrobial activity of *Tinospora cordifolia* was first assessed using ATCC reference strains. Ethanol extract showed zones of inhibition on these ATCC strains except *E. coli* strains 35218. Similarly, chloroform extract showed zones of inhibition on these ATCC strains except *E. coli* strain 35218 and *E. coli* strains 25922. However, the aqueous extract did not show any zone of inhibition in any of the ATCC strains (Table 1) (Figure 4).

After evaluating the effect of *Tinospora cordifolia* using ATCC strains, it was tested against MDR strains isolated from patient samples. The antibacterial activity of ethanol and chloroform extracts showed high antimicrobial effect against most of the tested bacterial isolates. A zone of inhibition was observed in *Enterobacter* spp., *E. coli*, *Acinetobacter baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus* using both ethanol and chloroform extracts, whereas aqueous extract showed antimicrobial effects only on *Klebsiella* spp. and *Enterobacter* spp. in clinical samples (Table 2) (Figure 5).

### Results of MIC<sup>23</sup>

Among 30 MDR isolates, 16 samples showed a zone of inhibition, of which 10 samples were further subjected for the determination of MIC. The concentration of stock extract was 600 µg/mL. The extract was diluted to 1.8 µg/mL with a dilution factor 1/320. The typical effect of ethanol and chloroform extracts of *Tinospora cordifolia* was observed till 1/40 dilution factors, i.e 15 µg/mL. The

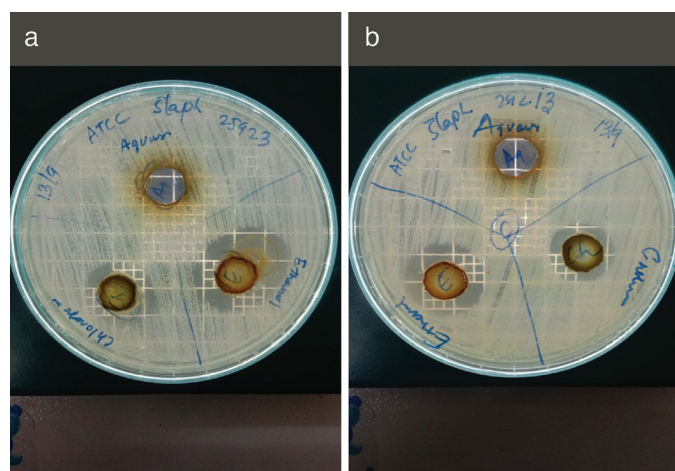
antimicrobial activities of *Tinospora cordifolia* extract were compared with ampicillin (500 µg/mL), which was used as positive control and DMSO was used as negative control (Figure 6). The results of the antibacterial activity of *Tinospora cordifolia* obtained using diffusion method and MIC are summarized in Table 3.

### Phytochemical Analysis of *Tinospora Cordifolia* Extracts

In the present study, the extract of *Tinospora cordifolia* revealed medicinally active and important bioactive constituents which are summarized in Table 4. Our extract showed the presence of alkaloids, carbohydrates, phytosterols, saponins, tannins, proteins, flavonoids, and terpenoids, whereas glycosides, gums & mucilage, and fixed oil were absent.

## DISCUSSION

The use of medicinal plants and their derivative constituents have curative and antimicrobial activity against bacterial agents. *Tinospora cordifolia* plays a far better role in combating clinically isolated pathogens and their resistance owing to the presence of bioactive components such as alkaloids, terpenoids, phytosterols, flavonoids, tannins etc<sup>24-26</sup>.



**Figure 4a, b.** Antimicrobial susceptibility testing by disc diffusion method for ATCC strains.

**Table 1. Zone of inhibition observed for the ATCC strains.**

S. no	Organism	Ethanol	Chloroform	Aqueous
1	<i>Staphylococcus aureus</i> (29213)	25 mm	16 mm	-
2	<i>Escherichia coli</i> (25922)	20 mm	-	-
3	<i>Staphylococcus aureus</i> (25923)	30 mm	24 mm	-
4	<i>Escherichia coli</i> (35218)	-	-	-
5	<i>Pseudomonas aeruginosa</i> (27853)	19 mm	15 mm	-

Our study was intended to explore the phytochemical properties and antibacterial activity of *Tinospora cordifolia* against MDR pathogens. Ethanol and chloroform extracts of *Tinospora cordifolia* exhibited greater extent of antibacterial activities against the clinical isolates compared with the aqueous extract. Our findings were in concordance with the findings of Yamuna and Febronia<sup>11</sup> in which the authors have shown that the ethanolic extract of *Tinospora cordifolia* contains potential antibacterial activity against urinary tract infection-causing bacterial strains. However, these findings differed from Mishra et al.<sup>27</sup>, whose results were *Tinospora cordifolia* extracts exhibiting variable inhibitory responses against pathogenic bacteria.

Similar to the study conducted by Kumar et al.<sup>28</sup> which showed that the crude extract of *Tinospora cordifolia* was effective against *E. coli* and other pathogens, the plant extract in our investigation has been shown to be beneficial against these pathogens. Such variations could be attributed to varying factors such as climatic, geographic, seasonal conditions, metabolic, extraction etc.

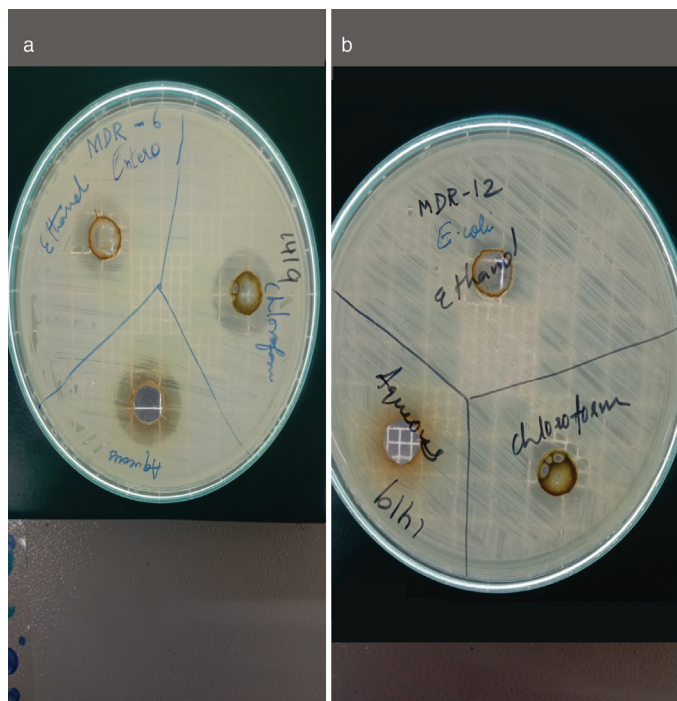
Since ancient times, medicinal plants have been valued for their natural and bioactive constituents for maintaining human health. Our phytochemical screening showed the presence of alkaloids, carbohydrates, phytosterols, saponins, tannins, proteins, flavonoids and terpenoids which strongly confirm its potential against

S. no.	Lab ID	Organism	Ethanol	Aqueous	Chloroform
1	MDR06	<i>Enterobacter</i> spp.	19 mm	21 mm	24 mm
2	MDR07	MRSA	10 mm	10 mm	10 mm
3	MDR08	<i>Staphylococcus aureus</i>	10 mm	Nil	Nil
4	MDR09	<i>Staphylococcus aureus</i>	20 mm	Nil	Nil
5	MDR10	<i>Klebsiella pneumoniae</i>	Nil	Nil	15 mm
6	MDR11	MRSA	Nil	Nil	Nil
7	MDR12	<i>Escherichia coli</i>	15 mm	Nil	17 mm
8	MDR13	<i>Acinetobacter baumannii</i>	15 mm	Nil	Nil
9	MDR15	<i>Klebsiella pneumoniae</i>	24 mm	25 mm	25 mm
10	MDR16	<i>Pseudomonas aeruginosa</i>	Nil	Nil	Nil
11	MDR17	<i>Klebsiella pneumoniae</i>	Nil	Nil	Nil
12	MDR18	<i>Klebsiella pneumoniae</i>	Nil	Nil	Nil
13	MDR19	<i>Escherichia coli</i>	Nil	Nil	Nil
14	MDR20	<i>Klebsiella pneumoniae</i>	Nil	Nil	Nil
15	MDR21	<i>Enterococci faecalis</i>	Nil	Nil	Nil
16	MDR22	MRSA	12 mm	Nil	10 mm
17	MDR23	<i>Klebsiella pneumoniae</i>	19 mm	Nil	22 mm
18	MDR24	<i>Klebsiella pneumoniae</i>	20 mm	Nil	23 mm
19	MDR25	<i>Klebsiella pneumoniae</i>	25 mm	Nil	24 mm
20	MDR26	<i>Escherichia coli</i>	Nil	Nil	Nil
21	MDR27	<i>Pseudomonas aeruginosa</i>	Nil	Nil	Nil
22	MDR28	MRSA	15 mm	Nil	10 mm
23	MDR29	<i>Klebsiella pneumoniae</i>	Nil	Nil	Nil
24	MDR30	<i>Escherichia coli</i>	Nil	Nil	Nil
25	MDR31	<i>Enterococci faecalis</i>	Nil	Nil	Nil
26	MDR32	<i>Escherichia coli</i>	Nil	Nil	Nil
27	MDR33	<i>Pseudomonas aeruginosa</i>	15 mm	Nil	13 mm
28	MDR34	MRSA	16 mm	Nil	15 mm
29	MDR35	<i>Klebsiella pneumoniae</i>	Nil	Nil	Nil
30	MDR36	<i>Escherichia coli</i>	18 mm	Nil	18 mm

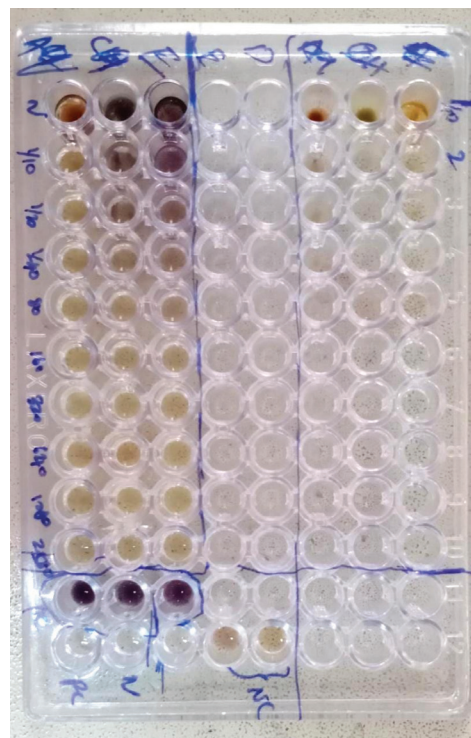
Nil: No one of inhibition, MRSA: Methicillin-resistant *Staphylococcus aureus*, MDR: Multidrug-resistant

the clinical isolates. The availability of such chemical constituents certainly establishes the effectiveness of this plant extract against MDR pathogens. These findings were comparable and were in concordance with the phytochemical screening done by Somalwar and Somalwar<sup>29</sup>, where they confirmed the presence of alkaloids, glycosides, sterols, oils etc. the plant extract.

However, a study by Bisset and Nwaiwu<sup>30</sup> reports protoberberine bases berbine and palmate as their main constituents. *Tinospora cordifolia* extracts were tested



**Figure 5a, b.** Antimicrobial susceptibility testing by disc diffusion method for the multidrug-resistant organisms.



**Figure 6.** Minimal inhibition concentration of ethanol, chloroform and aqueous extract of *Tinospora cordifolia* detection by MTT test.

MTT: 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide

**Table 3. Antimicrobial activity of *Tinospora cordifolia* (disc diffusion and MIC).**

S. no	Organism	Ethanol		Aqueous		Chloroform	
		Zone of inhibition	MIC	Zone of inhibition	MIC	Zone of inhibition	MIC
1	<i>Enterobacter</i> spp.	19 mm	1/40	21 mm	ND	24 mm	1/20
2	<i>Staphylococcus aureus</i>	10 mm	1/40	Nil	ND	Nil	1/20
3	<i>Staphylococcus aureus</i>	20 mm	1/40	Nil	ND	Nil	1/20
4	<i>Klebsiella pneumoniae</i>	Nil	1/40	Nil	ND	15 mm	1/20
5	<i>Escherichia coli</i>	15 mm	1/40	Nil	ND	17 mm	1/20
6	<i>Acinetobacter baumannii</i>	15 mm	1/40	Nil	ND	Nil	1/20
7	<i>Klebsiella pneumoniae</i>	24 mm	1/40	25 mm	ND	25 mm	1/20
8	<i>Pseudomonas aeruginosa</i>	15 mm	1/20	Nil	ND	13 mm	1/20
9	MRSA	16 mm	1/20	Nil	ND	15 mm	1/20
10	<i>Escherichia coli</i>	18 mm	1/20	Nil	ND	18 mm	1/20

1/40 dilution factor represents 15 µg/mL

1/20 dilution factor represents 30 µg/mL

ND: Not done, Nil: No one of inhibition, MRSA: Methicillin-resistant *Staphylococcus aureus*, MIC: Minimum inhibitory concentration

Table 4. Preliminary phytochemical screening of <i>Tinospora cordifolia</i> extract.					
S. no	Test	Observation	Chloroform extract	Ethanol extract	Aqueous extract
1.	<b>Alkaloids</b>				
	Mayer's reagent	+	+	+	+
	Dragendorff's reagent	+	+	+	+
	Hager's reagent	-	-	-	-
2.	Wagner's reagent	+	+	+	+
	<b>Carbohydrates</b>				
	Molisch's test	+	-	+	+
	Fehling's test	+	-	+	+
3.	Benedict's test	+	-	+	+
	<b>Glycosides</b>				
	General test	-	-	-	-
	Anthraquinone	-	-	-	-
4.	Cardiac	-	-	-	-
	Cyanogenetic	-	-	-	-
	<b>Phytosterols</b>				
5.	Salkowski test	+	+	-	-
	Lieberman Burchard's test	+	-	-	-
6.	<b>Saponins</b>	+	+	+	+
7.	<b>Tannins &amp; phenolic</b>	+	-	+	+
8.	<b>Proteins and free amino acids</b>				
	Biuret's test	+	-	-	+
9.	<b>Gums and mucilage</b>	-	-	-	-
10.	<b>Flavonoids</b>				
	Shinoda test	+	+	-	+
11.	<b>Terpenoids</b>	+	+	+	-
	<b>Fixed oil</b>	-	-	-	-

"+" indicate positive reaction; "-" indicate negative reaction

with the standard antibiotics, i.e. ampicillin (30 g/disc), and our negative control, which was solvent without the plant extract proved satisfactory. The standard antibiotics showed significant antimicrobial activity against the tested pathogens, which included Gram-positive and Gram-negative clinical isolates.

The antimicrobial activity of *Tinospora cordifolia* against the test organisms showed different zones of inhibition, indicating the susceptibility of the test organisms to the *Tinospora cordifolia* extracts. Factors such as the presence of secondary plant metabolites might be the reason for the growth inhibitory action of *Tinospora cordifolia*, which showed concordant readings with the standard reference strains. Our findings were similar to the study done by Taechowisan<sup>15</sup> from Thailand,

in which the author concluded that *Tinospora cordifolia* exhibits great antibacterial activity and may be useful for their medicinal functions. The results with the plant extracts of *Tinospora cordifolia* showed similar and comparable antibacterial activity with the reference strains as reported with other similar findings<sup>31</sup>.

Our plant extract had MIC values between 15-30 µg/mL, indicating a better antimicrobial susceptibility, in contrast to Mushtaq et al.'s<sup>32</sup> investigation, which had a MIC value of 500 µg/mL and showed very moderate antibacterial activity. These findings suggest that *Tinospora cordifolia* may be effective for treating infectious disorders brought on by organisms that are MDR, and may also point the way toward improving the patient access to healthcare.



A larger sample size and comparison with other medicinal plants could be further helpful in deriving effective conclusions against multidrug or total drug resistant clinical isolates.

## CONCLUSION

Our results suggest the presence of major bioactive compounds isolated from *Tinospora cordifolia* plant extract which play a crucial role in the antibacterial activity against clinical isolates. The highlight of the current study is the antimicrobial activity of this medicinal plant extract against MDR bacteria isolated from clinical samples.

*Tinospora cordifolia* is a versatile medicinal herbal plant with a wide range of abundant bioactive constituents. With newer drug development programs, modern drugs should be developed from *Tinospora cordifolia* and its compounds. Drug delivery systems should use this plant as a classical agent for anti-diabetic, anti-cancer, immunomodulatory, anti-oxidant, antimicrobial and anti-toxic resource for a better and exploratory therapeutic option in healthcare system.

## Acknowledgments

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## Ethics

**Ethics Committee Approval:** The study was conducted after Sri Venkateshwarra Medical College Hospital and Research Centre Institutional Ethics Committee clearance (No. 03/SVMCH/IEC- Cert/ June22, date: 19.07.2022) for two months from August 2022 to September 2022.

**Informed Consent:** The study doesn't require patient consent.

**Peer-review:** Externally and internally peer-reviewed.

## Author Contributions

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