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Phytochemical Screening and Antibacterial Potential of *Tinospora Cordifolia* against Highly Resistant Human Pathogen's

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Abstract

Tinospora cordifolia is an herbaceous vine of the family *Menispermaceae*. This plant is indigenous to the tropical areas and distributed throughout India, Myanmar and Sri Lanka. Phytochemicals investigation revealed crude and treated extracts of the *T. cordifolia* plants stem were contain more or less same type of chemical constituents using extract methanol, chloroform and petroleum ether. This plant stem extracted from various organic solvent were tested against some Gram-positive, Gram-negative *bacteria* and *fungi*. Selected human pathogens *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli* and *Fungi* .The total eight samples of the five bacteria were study for antibiotic susceptibility against standard antibiotics like Gentamicin. Antibacterial activity of crude extract was carried out using disc diffusion method and MIC of isolated compound was tested by broth microdilution method. Thus the present study suggested crude petroleum ether extract showed good antibacterial activity against tested microbes.

Keywords: *Tinospora cordifolia*, phytochemical, human pathogen, antibiotic susceptibility, disc diffusion method

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Introduction

*T. cordifolia (*Menispermaceae) commonly known as Guduchi in India (Patel, 2011). It is an important medicinal plant used in ayurvedic system of medicine. It is widely distributed in tropical parts of the country. The plant family Menispermaceae consists of about 70 genera and 450 species that are found in tropical lowland regions (Abhimanyu Sharma *et al.*, 2010). They are generally climbing or twining, rarely shrubs. Leaves are alternate or lobed, flowers small cymas, seeds usually hooked or deiform. *Tinospora* is one of the important genera of the family, consisting of about 15 species. Some medicinally important species includes *T. Cordifolia, T. Malabarica, T. Tementosa, T. Crispa, T. Uliginosa, etc T. Cordifolia* (wild) Miers ex Hook. F and Thoms belonging to the family Menispermaceae is a large deciduous climbing shrub found throughout India and also in Srilanka, Bangladesh and China. Common name are …..

Hindi - Giloya, Guduchi
English - Tinospora
Tamil - Shindilakodi

The stems are freshly and roots are long thread like, aerial, arise from twigs. Bark is thin, grayish or creamy white in color, when peeled fleshy stem is exposed. The seeds are curved, pea sized. The extract obtained from the root is pure white whereas that obtained from the stem may be slightly grayish. The freshly prepared Guduchi extract has a good taste. Furthermore; many drugs had their origin in plant extract. There are many herbs, which are predominantly used to treated cardiovascular problems (Veeramuthu *et al.*, 2012) liver disorders, central nervous system, and digestive metabolic disorders. Diabetes (Singh *et al.*, 2003), jaundice, stomachache, improve the immune system and various types of fever. It helps to remove urinary stones and reduce blood urea (Joshi *et al.*, 2013).

Plants are invaluable sources of pharmaceutical products and plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases (Olalde Rangel *et al.*,2005) (Sankhala *et al.*, 2012). *T. califora* shows significant bactericidal activities .It improves bacterial clearance as well as improves phagocytic and intracellular bactericidal capacities of neutrophils. It also stimulates macrophage action. As a result it stimulates immune system of body (Thatte *et al.*, 1992). Despite the wide availability of clinically useful antibiotics, a continuing search for new anti-infective agents remains indispensable because some of the major antibacterial agents have considerable drawbacks in terms of limited antimicrobial spectrum or serious side effects (Antara sen *et al.*, 2012). The negative health trends call for a renewed interest in infectious disease in the medical and public health communities and renewed strategies on treatment.

Materials and methods

Collection of Plant Materials

The stem of the plant *Tinospora califora* collected from various part of the Kanyakumari district, Tamilnadu, India. The stems were cleaned and wet dried for three weeks and grounded into fine powder then these samples in powdered form is used for extraction (Setti and Micetich, 1998).

Preparation of plant extract

The dried powdered stem of *T. califora* was allowed to pass through SS sieve (20 mesh). It was defatted by treating with Petroleum ether (60-80^{o}C) and then extracted to exhaustion (soxhlet) with various solvents like Methanol, chloroform and Petroleum ether the excess solvent was removed under vacuum to get the solid mass.

Standardization

Physico chemical parameters were determined as per guidelines of WHO. Total ash,acid insoluble ash, water soluble extractive value, alcohol soluble extractive value, heavy metals were determined by using standard procedures

Soxhlet Extraction

The powdered plant materials (25g) was extracted with methanol, chloroform and petroleum ether at 40-80˚C depending upon the evaporation point of the solvent by soxhlet extraction. The extraction was carried out using solvent of increasing polarity from petroleum ether, chloroform and methanol respectively .The extraction was carried out for six hours. After the complete extraction the extract was taken in watch glass and kept for air drying for the evaporation of the solvent, finally dry powder was remaining in the watch glass, the dry powder was collected and stored for the further studies. Extraction procedures performed repeatedly to procure reasonable quantity of plant material for further research aspects.

Phytochemical Screening of *T. califora*

Crude extract in methanol ,chloroform and petroleum ether of the plants were subjected to qualitative phytochemical screening for protein, carbohydrate, saponin, tannin, glycoside, alkaloids, flavanoids, terpenoids, and carbohydrate according to the method .

Qualitative Phytochemicals analysis

The hydroalcoholic and aqueous extracts of *T. califora* were subjected to different chemical tests for the detection of phytoconstituents such as carbohydrates, glycosides, alkaloids, proteins, tannins, phenolics, saponins, flavonoids, triterpenoids and steroids (Harbone 2005).

1. Tests for carbohydrates:

***Fehling's Test*:** 1 ml. Fehling's A solution and 1 ml. of Fehling's B solution were mixed and boiled for one minute. Then the equal volume of test solution (extract) was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, then brick red precipitate was observed.

2. **Tests for proteins:**

***Xanthoproteic Test*:** To the small quantity of extract 1ml. of conc. H2SO4 was added. This resulted in the formation of white precipitate which on boiling turned yellow. On addition of NH4OH, yellow ppt. turned orange.

3. **Tests for glycosides:**

***Keller-Killiani Test*:** To 2 ml of the extract, glacial acetic acid, one drop 5% $FeCl_3$ and conc. H_2SO_4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

4. **Test for steroids:**

***Leibermann's Reaction*:** 3 ml. of extract was mixed with 3 ml. of acetic anhydride. The test solution was then heated and cooled. A few drops of conc. H_2SO_4 were added to the test solution. Appearance of blue color shows the presence of sterols.

5. **Tests for alkaloids:** The extract was evaporated in a test tube. To the residue dilute HCl was added, shaken well and filtered. With the filtrate following tests were performed:

***Mayer's Test*:** To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

6. **Tests for flavonoids:**

***With Lead Acetate*:** To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoid.

7. **Tests for Tannins and Phenolic compounds:**

***Lead Acetate Test*:** On addition of lead acetate solution to the extract white precipitate appeared.

8. **Test for saponins:**

***Foam Test*:** Drug extract was shaken vigorously with water. No persistent foam was formed

9. **Test for triterpenes:** To the extract chloroform and conc. H_2SO_4 was added. Appearance of red color indicated the presence of triterpenes.

Test Microorganisms

Present investigation was carried out with human pathogenic bacteria .Selected human pathogens *Staphylococcus aureus,*

Pseudomonas aeruginosa, *Klebsiella pneumonia*, *Escherichia coli* and *fungal* .The bacterial microorganisms were clinical isolates.

Determination of antibacterial activity *(Disc diffusion method)*

Screening of antibacterial activity was carried out by plate disc method (Harbone *et al.*,1984) **.**The disc with test compound placed on the upper surface of sterilized Muller Hinton plate that had been inoculated with the test organism (using a sterile swab) and air dried to remove the surface moisture. The thickness of MH medium was kept equal in all Petri plates and the standard disc (Gentamycin) was used in each plate as control. The plates were incubated for 24 hrs at 37°C for bacteria and 48 hrs at 27°C for fungi. Zones of inhibition were recorded in millimeters. The research was conducted in replicates of 3 and the mean value is presented.

Minimum Inhibitory concentration:

The MIC of the extracts was determined according to Elizabeth *et al.* (1999).The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was resolute from readings on the culture plates after incubation. The most common methods are the tube dilution method and agar dilution methods. Serial dilutions are made of the products in bacterial and fungal growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard examine for antimicrobials. Minimum inhibitory concentrations are significant in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against a microorganism. Clinically, the minimum inhibitory concentrations are used not only to determine the quantity of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the prospect for microbial resistance to specific antimicrobial agents.

Determination of MIC

The minimum inhibitory concentrations (MIC), MBC and MFCs were performed by a serial dilution technique using 96-well microtiter plates. The different plant extracts viz. Methanol, Chloroform and Petroleum ether, Aqueous were taken (1 mg/ml) and serial dilution of the extract with Luria broth for bacterial culture and for fungus, MH medium with respective inoculums were used. The micro plates were incubated for 72 hours at 28 ^{o}C, respectively. The lowest concentrations lacking visible growth (at the binocular microscope) were clear as MICs.

Phytochemicals

The results of exacted value in extract methanol, chloroform, and petroleum ether are shown in Table -1. Alkodise were present in Methanol extract of plant ,while absent of chloroform and petroleum ether (Mallikharjuna *et al*.,2007). Alkaloids are heterocyclic indole compounds which have proved to be having pharmacological properties. Primary metabolites like carbohydrate and protein were detected in both chloroform extract of plant, protein absent in methanol extract and carbohydrate absent in petroleum ether plant extract. Steroids were present only in chloroform crude extract. Methanol and chloroform were showed positive results for glycosides expect Petroleum ether .Absence of flavanoids in methanol and petroleum ether, while presence of chloroform curde plant extract. Flavonoids and tannins are phenol compounds and plant phenolics are a major group of compounds that are as primary antioxidant or free radical scavengers [Sivakumar *et al.*, 2010]. Flavonoids show anti allergic, anti-inflammatory, anti- cancer activity [Yamato *et al.*, 2002]. Tanninsa Phenoliccom was absent plant all extract. Soponin and triterpen present only chloroform extract, while absent of other extract.

Table 1: Preliminary Phytochemical test of *Tinospora cordifolia stem extract with* Petroleum ether, Chloroform and Ethanol.

S. No	Test sample	Methanol extract	Chloroform extract	Petroleum ether
1	Alkaloids	+	-	-
2	Flavanoids	-	+	-
3	Protein	-	+	+
4	Tannins Phenoliccom	-	-	-
5	Soponin	-	+	-
6	Triterpen	-	+	-
7	Steroids	-	+	-
8	Carbohydrates	+	+	-
9	Glycodies	+	+	-

Present : +, Absent : -

Antimicrobial activity

Results of comparative antimicrobial activity of chloroform, methanol extract and petroleum ether extracts of all plants are recorded in Table 2. At presently commercial antibiotic were using Gentamycin compare with crude extract of plant high resistance 08 microbes. Methanol extract disc resistance with microbe E.coli (Eey) 09mm other microbes are not resistance with methanol (fig: 1). *Staphylococcus aureus* (sample V) resistance only Petroleum ether crude extract .In the present study high resistance inhibition 14mm (fig: 2). *sample-Xx Klebsiella pneumonia* were resistance with chloroform plant crude extract inhibition 10mm (fig:3). One strong observation was distinguished in present study was that in the plants *T. cordifolia* and Petroleum ether extract showed higher antibacterial activity than chloroform and Methanol extract (fig:5). Uddin *et al.*, 2011. Zainab *et al.*, 2013 also reported petroleum ether extract of *T. cordifolia* exhibit high antibacterial activity .All strain of bacteria was susceptible to positive control Gentamycin (15mm) more inhibitory all pathogens with the all curd extract and sterile disc 100 % as negative control was not inhibiting any bacterial strain.

Minimum Inhibihitory Concentration (MIC)

The analysis of Table 3 shows the antibacterial potentiality of the extract of the whole plant. The pattern of drug resistance towards the extract is also revealed against various clinical microorganism treated in our study. Methanol plant extract inhibit s. sample (V) *S. aureus* (200µl, 250 µl) sample (Xx) *K. pneumonia* (200 µl, 250 µl) expects *E.coli.* chloroform plant extract inhibit only (E.ey) *E.coli* (200 µl, 250 µl).petroleum either highly inhibit *E.coli* (100 µl,150 µl,200 µl,250 µl)and *K. pneumonia* (200 µl, 250 µl) fig:-4.

The result suggests that the petroleum either extracts from whole plant of *T. cordifolia* exhibited significant dose dependant antibacterial activity against the tested microbes. Deepak Gahlawat *et al.,* (2013) the extract was found to be inhibiting the microorganism in a dose dependent manner and the activity was comparable with a dose of Gentamicin. To justify the traditional uses of plant we have scientifically screened the whole plant of *T. cordifolia* for antimicrobial potentiality.

Plant sample	Solvent Used	Bacterial pathogen used and zone of inhibition of mean ± SD in mm.							
		H	Eey	Yy	Ym	Aam	Xx	Cv	V
T. cordifolia stem	Methanol	-	9	-	-	-	-	-	-
	Petroleum ether	-	-	-	-	-	-	-	14
	Chloroform	-	-	-	-	-	10	_	_
Positive control	Methanol + Gentamycin	18	20	16	21	16	25	30	16
	Petroleum ether + Gentamycin	20	18	18	18	24	20	20	21
	Chloroform + Gentamycin	22	18	20	26	22	24	21	22

Table 2: Antibacterial activity of *Tinospora cordifolia stem* disc diffusion method

Sample*(Yy,Aam and V)Staphylococcus aureus* , Sample *(Cc)Pseudomonas aeruginosa*, sample-*(H,Xx) Klebsiella pneumonia*, Sample (Eey)- *Escherichia coli, Sample (Ym)fungai*

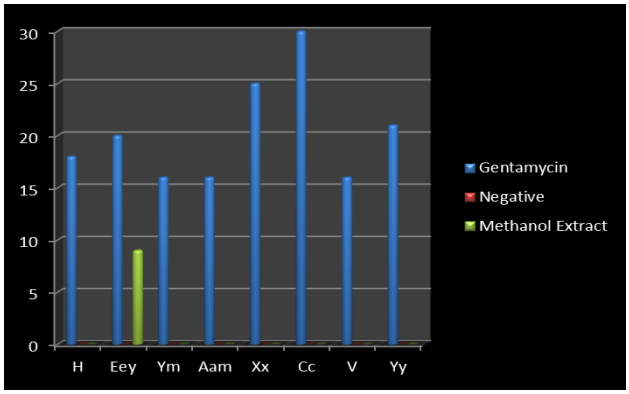

Figure 1: Antibacterial activity of *Tinospora cordifolia stem* disc diffusion method methanol extract.

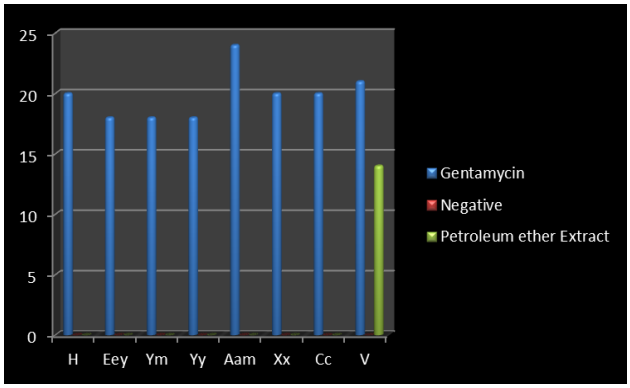

Figure 2: Antibacterial activity of by *T. cordifolia stem* disc diffusion method petroleum ether.

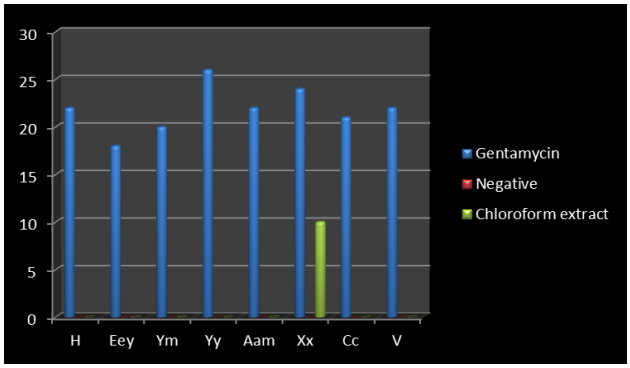

Figure 3: Antibacterial activity of *Tinospora cordifolia stem* disc diffusion method chloroform extract.

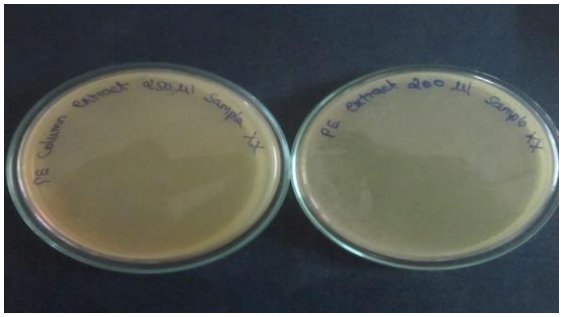

Figure 4: MIC petroleum ether plant extract.

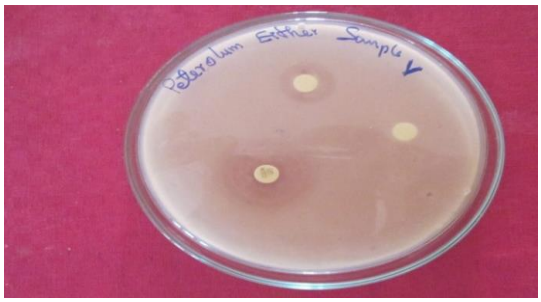

Figure 5: Petroleum ether sample Antibacterial activity

Table 3: Determination of MIC of methanol, petroleum either and chloroform extract of whole plant of *Tinospora cordifolia* against various human bacterial

Samples	Conc. .of ***plant extract (µl).***	Methanol	Petroleum either	Chloroform
		MIC growth of human colonies	MIC growth of human colonies	MIC growth of human colonies
Escherichia coli (Eey)	50	600	150	300
	100	560	30	160
	150	556	00	60
	200	540	00	00
	250	500	00	00
Staphylococcus aureus (V)	50	365	700	800
	100	190	659	750
	150	108	575	680
	200	30	450	500
	250	00	400	458
Klebsiella pneumonia (Xx)	50	280	350	1000
	100	40	89	850
	150	18	40	669
	200	00	00	478
	250	00	00	500

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