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ORIGINAL ARTICLE

Phytochemistry and assessment of *in vitro* antibacterial activity of *Tinospora cordifolia* hydroethanolic extract

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ABSTRACT
The present investigation was carried out to study the phytochemistry and to evaluate the anti-bacterial activity of hydroethanolic extract of Tinospora cordifolia (HETC) in-vitro. Phytochemical screening of the extract revealed the presence of various phytoconstituents, viz, alkaloids, steroids, reducing sugars, glycosides and proteins while anthraquinones, flavonoids, resins, terpenes, tannins and saponins were absent. Proteins (86.27 µg/mg of extract) and phenols (70.12 µg/mg of extract) were found in higher concentrations. SDS-PAGE analysis of the extract revealed 28 bands of protein ranging from 14.4 KDa to 250.0 KDa. FTIR spectrum indicated the presence of amide/amine groups having N-H stretching vibrations. HETC showed antibacterial activity against gram positive bacteria viz., Bacillus and Staphylococcus. It can be concluded from the study that high protein content, phenols and alkaloids in the plant might be responsible for its antibacterial activity in gram positive bacteria.
Keywords: *Tinospora cordifolia hydroethanolic extract (HETC), Phytochemical screening, SDS-PAGE analysis, FTIR analysis.*

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INTRODUCTION

Tinospora cordifolia (family *Menispermaceae*) commonly called as Giloy or Indian Bitter is a drug of Indian System of Medicine (ISM) and is being used as a medicine in fever, diabetes, dyspepsia, urinary and gastrointestinal ailments [10]. The plant is distributed in tropical region of India upto 1200m above mean sea level from Kumaon region of Uttarakhand to Assam and in Deccan and Konkan regions [2]. It is a climbing shrub, stem is soft wooded, porous, dry, cylindrical and contain long filiform aerial roots expanding from branches and bitter in taste [1]. The pharmacological significance of *Tinospora cordifolia* is attributed to various bioactive compounds found in the plant extract mainly Terpenoids (tinosporide, furanoid diterpene, cordifoliosides), alkaloids (tinosporin, berberine, choline, palmatine), lignans, steroids (giloinsterol, ß- sitosterol) and others like giloin and giloinin [2,4]. Commercial formulations of *Tinospora cordifolia* such as *Guduchi Ghana* and *Pepticare* possess immunostimulatory action and anti-ulcer action, respectively.

The hydroalcoholic extract of *Tinospora cordifolia* have been reported to be rich in flavonoids, alkaloids and glycosides [11] and has been shown to exhibit antibacterial property against certain bacteria such as *Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Staphylococcus epidermidis* etc [3].

Therefore, the present study was conducted to explore of the possibility of using *Tinospora cordifolia* extracts as a potential plant for common bacterial infections in humans and animals and for the development of herbal formulations in future.

MATERIALS AND METHODS

The study was conducted in the Department of Biophysics, Department of Microbiology, College of Basic and Animal Sciences and Department of Pharmacology & Toxicology, College of Veterinary and Animals Sciences, G B Pant University of Agriculture and Technology, Pantnagar in the year 2017.

Plant Material

Tinospora cordifolia stems were collected, identified and authenticated from Medicinal Research and Development Centre (MRDC), Pantnagar. The stems were cut into small pieces of 0.5-1 cm length and shade dried. The dried stems were ground to fine powder for extract preparation.

Preparation of the extract

50% hydroethanolic extract was prepared by extraction of ground powder in 50% ethanol. The extract was prepared by cold extraction as per the method described by Pande, [4]. 54 gram of powdered stem was soaked in 540 ml (1gm/10ml of hydroethanolic solution) of 50% hydroethanolic solution with continuous stirring for 24 hours at 37°C. The mixture was filtered through eight layers of muslin cloth and centrifuged to separate the supernatant. The final extract was produced after drying the filtrate in fan incubator at 35°C. The dried extract was collected, percent yield was calculated and then the extract was kept in air tight bottles at 4°C till further use.

Phytochemical analysis of the plant extract

Qualitative chemical analysis of hydroethanolic extract of *Tinospora cordifolia* was done to detect major phytochemical groups viz., alkaloids, anthraquinones, carbohydrates, proteins, reducing sugars, flavonoids, saponins, sterols, tannins, terpenes and glycosides [5,6].

Quantitative chemical analysis of hydroethanolic extract of *Tinospora cordifolia* was done to detect primary metabolites viz., carbohydrates, starch, proteins and total phenols [14, 15].

SDS-PAGE of hydroethanolic extract of plant

Protein extraction

54 grams dried fine powder from stem of *Tinospora cordifolia* was soaked in 540 ml of 50% ethanol. It was kept at room temperature with continuous mixing for 24 hours and then filtered using Whatman paper no. 40. Filtrate was stored at 4°C. Protein precipitation was done by 90% ammonium sulphate saturation. Filtrate was stirred using magnetic stirrer and saturated ammonium sulfate was added slowly while stirring to bring final concentration to 50% saturation. The saturated filtrate was stored at 4°C overnight and centrifuged at 10,000 rpm for 30 minutes next day. Supernatant was decanted off and pellet was resuspended in 1X-PBS.

One dimensional SDS-PAGE analysis was performed by the method described by Kumar and Pandey, [8]. For sample preparation, approximately 50 µg of protein sample was taken with 2x buffer in 1:1 ratio. This was boiled in boiling water bath for 5 min. The sample was centrifuged at 10,000 rpm for 10 min. The samples were loaded with help of micropipette. The gel was run at 2-3 mA at constant voltage of 80 Volts till the samples was in stacking gel and after that voltage was raised to 100 volts. The gel was allowed to run till the dye reach 0.5 cm from the lower edge of gel. After completion, gel was placed in a tray containing the fixing solution for 30 min. After that, the gel was left in staining solution overnight**.** Next day, gel was taken out and kept in destaining till the bands were clear.

FTIR analysis of hydroethanolic extract of plant

FTIR spectrum of hydroethanolic extract of *Tinospora cordifolia* was analyzed by attenuated total reflection (ATR) method. Attenuated total reflection (ATR) is a sampling technique used in conjunction with infrared spectroscopy which enables samples to be examined directly in liquid state and uses a property of total internal reflection. A beam of infrared light is passed through the ATR crystal in such a way that it reflects at least once off the internal surface in contact with the sample. The spectrum was recorded in the range of 1000-4000 cm^{-1}. Before proceeding further nitrogen purging was performed till the background peak of CO_2 was completely disappeared. This eliminates background noise in the final spectra. Infra-red spectra of 1 ml ethanol was recorded and treated as a background for the sample as extract of *Tinospora cordifolia* was prepared in ethanol. After that 1ml of sample was poured on to the chamber plate and allowed to transmit infra-red radiations through it. After 32 repeated cycles FTIR spectra of hydroethanolic extract of *Tinospora cordifolia* was recorded and analyzed.

Antibacterial activity of hydroethanolic extract of *Tinospora cordifolia* on bacterial strains

The plant extract was screened against four bacterial strains, *Escherichia coli* (ATCC 25922), *Salmonella* Typhimurium (ATCC 23564), *Bacillus subtilis* and *Staphylococcus aureus.* Penicillin (10mcg/disc) was taken as the standard antibiotic for gram positive bacteria whereas streptomycin (10mcg/disc) for gram negative bacteria.

Stock solution (100 mg/ml) was made in 50% ethanol, filter sterilized and working solutions of 25 µg/ml and 50µg/ml concentrations were made. The filter paper discs (6 mm) were impregnated with 20µl each

of the working solution using a pipette. These were dried in the hot air oven at 50° C for 24 hours for complete drying.

The antibacterial activity of the extract was evaluated using the *in vitro* agar disc diffusion method against selected pathogenic bacteria [7]. Fresh nutrient agar plates were streak cultured with bacterial strains and incubated at 37° C for 24 hours. Microbial culture was taken and Mueller Hinton agar plates were streaked by using a sterile swab streak covering the entire plate. Standard antibiotic and extract impregnated discs were placed on the streaked plate and kept in incubator at 37° C for 24 hours. The zone of inhibition was measured to determine antibacterial activity.

RESULTS AND DISCUSSION

The phytochemical analysis of extract revealed the presence of alkaloids, proteins, reducing sugars, glycosides and steroids and the absence of anthraquinones, flavonoids, saponins, tannins, terpenes and resins as mentioned in table 1.

Carbohydrate concentration was found to be 7.5µg/mg, starch concentration 8.69µg/mg, protein concentration 86.27µg/mg and total phenol 70.12µg/mg of hydroethanolic extract (Table 2). It was observed that the primary metabolites present in hydroethanolic extract of *Tinospora cordifolia* were present in varying quantity. Carbohydrates and starch were present in lower quantity whereas protein and phenolics content were present in very high quantity.

Table 1: Qualitative phytochemical analysis of *Tinospora cordifolia.*

S.No	Phytoconstituents	Method	Hydroethanolic extract of *Tinospora cordifolia*
1.	Alkaloids	Wagner's test	Present
2.	Proteins	Biuret test	Present
3.	Reducing sugar	Benedict test Benedict test	Present
4.	Glycosides	Salkowski test	Present
5.	Steroids	Bentranger's test Shinoda	Present
6.	Anthraquinones	test	Absent
7.	Flavonoids	Foam test	Absent
8.	Saponins	Ferric chloride test	Absent
9.	Tannins	Salkowski test Turbidity	Absent
10.	Terpenes	test	Absent
11.	Resins		Absent

Table 2: Quantitative phytochemical analysis of hydroethanolic extract of *Tinospora cordifolia*

S.No	**Primary metabolites**	**Hydroethanolic extract of *Tinospora cordifolia***
1.	Carbohydrate	7.5 µg/mg
2.	Starch	8.69 µg/mg
3.	Protein	86.27 µg/mg
4.	Total Phenols	70.12 µg/mg

Table 3: Antibacterial activity of *Tinospora cordifolia* extract against gram-positive bacteria

S. No.	Test organism (Bacteria)	Diameter of Zone of Inhibition (in mm) Mean±SE			
		HETC		Penicillin	Streptomycin
		25µg/ml	50µg/ml		
1.	*Bacillus subtilis*	7.90±0.06	18.06±0.05	18.06±0.06	--
2.	*Staphylococcus*	9.00±0.05	16.96±0.05	18.03±0.08	--
3.	*Escherichia coli*	--	--	--	9.33±0.33
4.	*Salmonella* Typhimurium	--	--	--	4.20±0.11

HETC – Hydroethanolic extract of *Tinospora cordifolia*

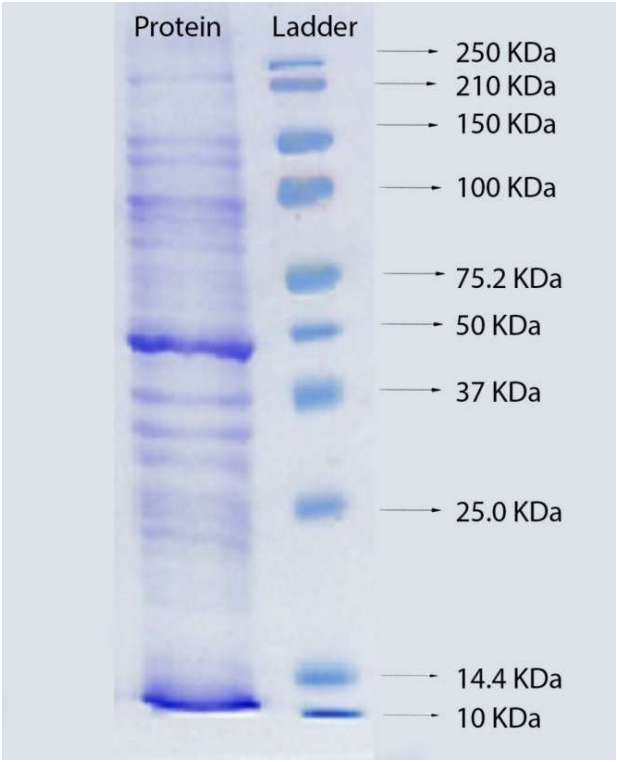


Fig 1: SDS-PAGE of hydroethanolic extract of *Tinospora cordifolia.*

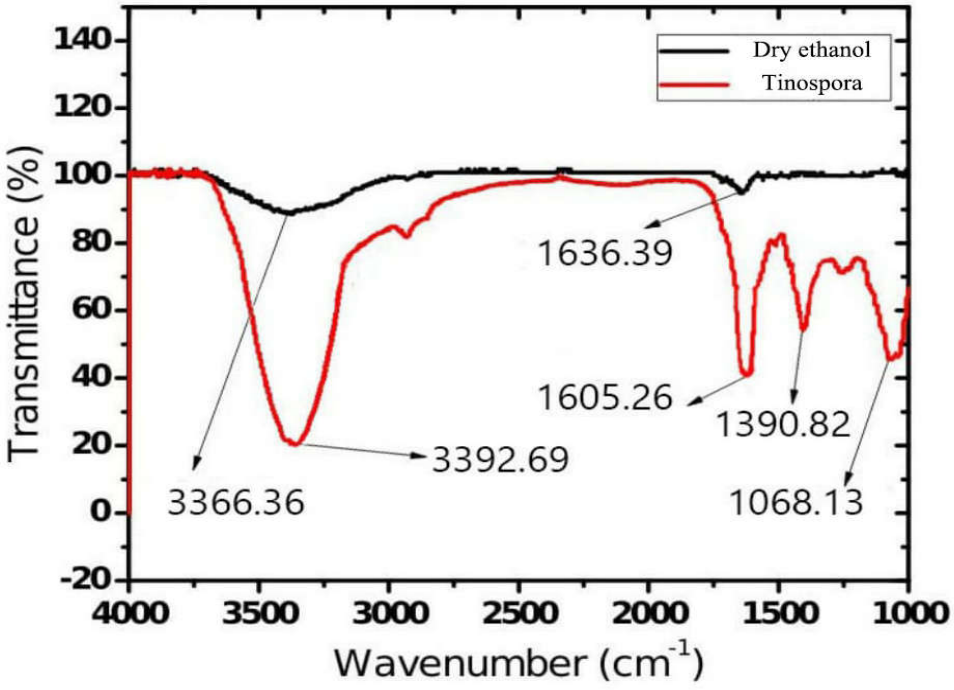
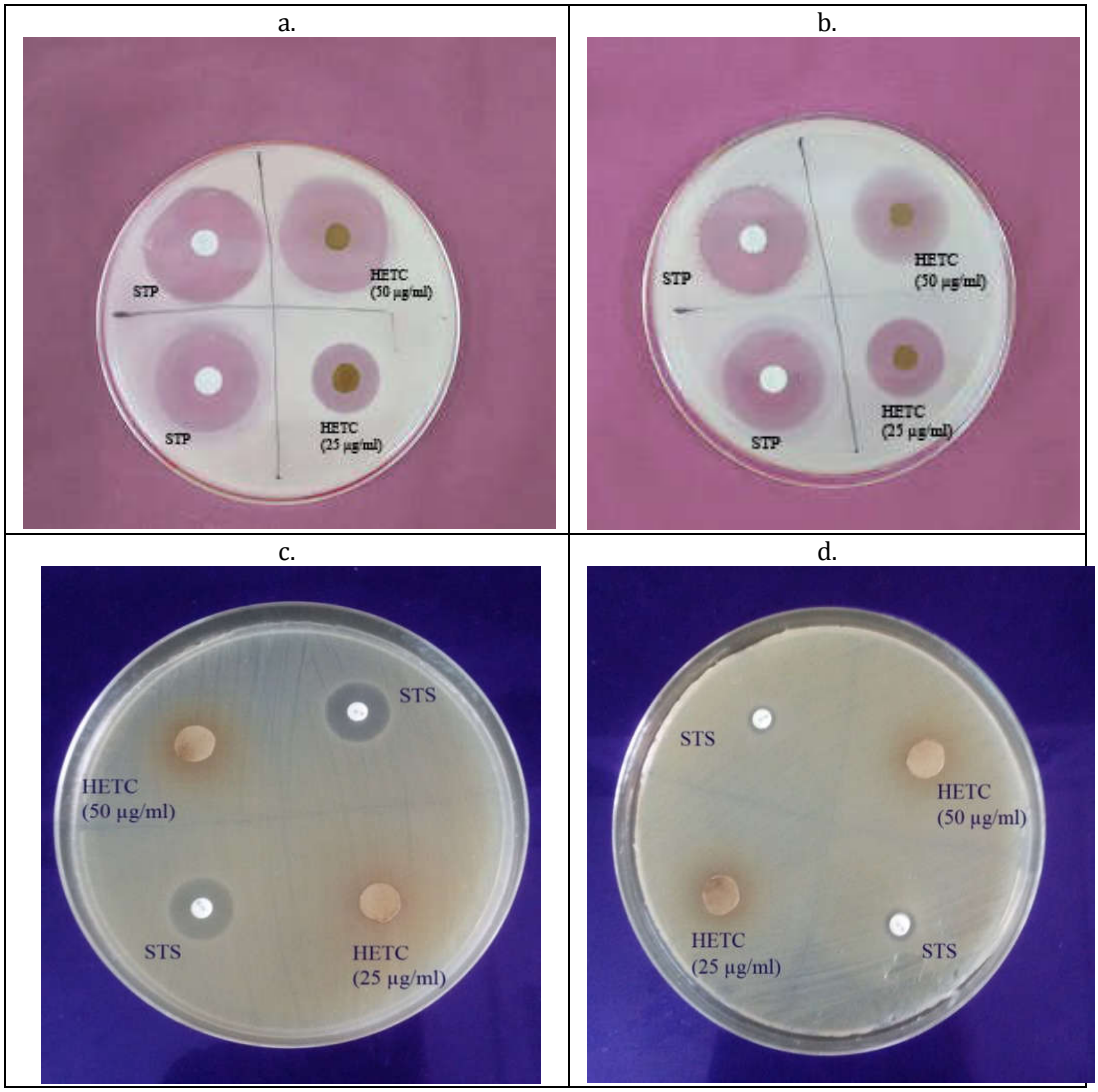


Fig 2: Fourier Transform Infrared Spectroscopy of hydroethanolic extract of *Tinospora **c**ordifolia*



STS: Standard Streptomycin STP: Standard Penicillin

Fig 3: a. Antibacterial activity of *Tinospora cordifolia* extract against *Bacillus subtilis.*
b.Antibacterial activity of *Tinospora cordifolia* extract against *Staphylococcus.*
c.Antibacterial activity of *Tinospora cordifolia* extract against *Escherichia coli.*
d.Antibacterial activity of *Tinospora cordifolia* extract against *Salmonella* Typhimurium.

SDS-PAGE analysis of the extract revealed approximate 28 bands of proteins ranging from 14.4KDa to 250.0 KDa. Protein profiles further showed variability on the basis of presence or absence and intensities of protein bands with banding pattern. Overall out of 28 protein bands, molecular weights 18.5 KDa to 250KDa showed same protein band pattern in the stem extract with varying intensities. Intensity of band shows that ample amount of protein is present in the sample.

In FT-IR spectra, prominent transmission peak was found at 3392.69 cm^{-1}, 1605.26 cm^{-1}, 1390.82 cm^{-1} and 1068.13 cm^{-1} for the extract and no significant peak was found in dry ethanol, (fig 5). The strongest absorption band was assigned to $-NO_2$ (at 1605.26 cm^{-1} and 1390.82 cm^{-1}) of aliphatic nitro group having O-N stretching vibrations. Bands originating from free water or alcohol (hydroxyl group) were seen at approximately 3300 cm^{-1} and 1080 cm^{-1}, as well as a medium band was also seen at 1605.26 cm^{-1} indicated the presence of amide/amine groups having N-H stretching vibrations. Since, the presence of alkaloids in hydroethanolic extract of *Tinospora cordifolia* stem has been reported by various researchers, it can be quite possible that the amide/amine groups found in FT-IR spectra are a part of those alkaloids.

Hydroethanolic extract of *Tinospora cordifolia* showed significant antibacterial activity at 50µg/ml against *Bacillus subtilis* (zone of inhibition - 18.06±0.05) and *Staphylococcus* (zone of inhibition - 16.96±0.05) in comparison to Penicillin having zone of inhibition of 18.06±0.06 for *Bacillus subtilis* and 18.03±0.08 *Staphylococcus*. (Table 2 and Table 3). However, the extract did not show antibacterial activity

against *Escherichia coli* and *Salmonella* Typhimurium at both concentrations. The antibacterial activity can be due to the presence of alkaloids, phenols and proteins in the extract. Plants synthesize different types of natural products, including phenolics, alkaloids and free amino acids, which directly or indirectly participate in the defence mechanism against pathogens phenols exhibit effective antibacterial activity owing to their ability to form complexes with extra cellular and soluble proteins and to complex with bacterial cell walls leading to the death of the bacteria [12, 13].

Since the presence of zones of inhibition in the antibacterial activity assay indicates that the hydroethanolic extract of *Tinospora cordifolia* has antibacterial components, which was further confirmed by the presence of total phenol, alkaloids and proteins. Thus, *Tinospora cordifolia* may represent as a safe antibacterial compound for common bacterial pathogens.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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