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Study of phytochemical and antibacterial activity of leaves extract of *Tinospora cordifolia* (Willd.) Miers ex Hook

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Abstract
The aim of the study was to investigate the leaves extract of traditional medicinal plant *T. cordifolia* for qualitative estimation of phytoconsituents and subsequently determine its antibacterial and antioxidant activity to authenticate its use in traditional medicines. The leaf extracts of *T. cordifolia* expressed the presence of several phytochemicals *viz.*, alkaloids, glycosides, flavonoids, steroids, tannins, terpenoids, saponins and sugars. The methanolic extract displayed the presence of highest phytochemical compounds. It may be due to the higher solubility of active components in this solvent as compared to other solvents. Results suggest that the methanol, chloroform and ethyl acetate extract have substantial antibacterial activity against tested bacterial species. Methanolic extract showed noteworthy antioxidant potential compared to other solvents. The investigation further propose that the metabolites present in leaf tissue of *T. cordifolia* can be potential source of novel natural antibacterial and antioxidant agents and has prospective applications in food industry as an antioxidant.

Keywords: antibacterial, antioxidant, solvents extracts, *Tinospora cordifolia*

Introduction
Medicinal plants are considered as green gold owing to their invaluable contribution to the health care and well-being of human societies. The use of traditional medicine and medicinal plants on a normative basis to cure ailments and maintenance of good health has been widely observed in most developing countries since prehistoric times. The Indian system of medicine itself uses more than 8000 species of medicinal plants (Tripathi and Tripathi, 2003) [1].
Tinospora cordifolia (Willd.) Miers ex Hook. F., commonly named as Giloy, or Heart- leaved Moonseed is a genetically diverse, large, deciduous climbing shrub belonging to the family Menispermaceae (Anonymous, 2003; Aima, 2003 and Vaidya, 1994) [2-4] using in folk and Ayurvedic medicine, distributed throughout the tropical subcontinent and China, ascending to an altitude of 3000 m. *T. cordifolia* finds a special mention for its use in folk medicine in different parts of the country (Singh *et al.* 2003) [5]. Almost all parts of this plant are documented to be useful in ethno botanical surveys conducted by ethno botanist (Jain, 1991) [6]. Thus, the plant is considered as one of the most divine herbs in Ayurvedic medicine for its immense medicinal properties such as antidiabetic, antiperiodic, antispasmodic, anti-inflammatory, antiarthritic, antioxidant, antiallergic, antistress, antileprotic, antimalarial, hepatoprotective, immunomodulatory, and antineoplastic activities (Khosaand Prasad, 1971; Asthana *et al.* 2001; Rai, 1996; Pendse *et al.* 1997; Nayampalli and Desami 1986 and Mehra and Puri, 1969) [7-12].
The information regarding the *in vitro* antioxidative potential and antibacterial activity of *T. cordifolia* is sparse. Moreover, the extraction and further characterization of bioactive compounds from plants depend mainly on the solvents used for extraction (Anonymous, 2003) [2]. Hence, in the present investigation, we work out the antioxidant potential along with the antibacterial activity of leaves extract of *T. cordifolia* using different solvents for phytoconstituents extraction so that, the results of the study can be further use for screening and characterization of active compounds of this plant and validate its use in folk medicine.

Materials and Methods
Plant Material
Fresh plant material (leaves) of *T. cordifolia* was collected from the Botanical garden of Govt. Science P.G. College, Rewa (M.P.). Leaves samples were collected during summer season (April to August). Since, *T. cordifolia* is a vine, all plants examined in this study used neem trees (*Azadirachta indica*) as support. The samples were brought to the laboratory in sealed plastic bag and stored at 4°C.

Extraction Procedure
Grinding of selected plant materials
The plant material leaves were taken and washed with running tap water thoroughly to remove soil particles and other contaminants followed by rinsed with distilled sterilized water. Leaves were dried at 37°C for 72 h. Exposure to sunlight was avoided to prevent the loss of active constituents. After drying, the plant material was cut into pieces and ground to powdered form. The powdered plant material was taken for extraction procedure.

Extract Preparation
Air-dried and coarsely powdered plant was extracted for 8 h with different solvents (diethyl ether, ethyl acetate, chloroform, methanol, and water) in Soxhlet apparatus. Then, the extract was filtered and allowed to evaporate. The

dried extract is dissolved in 10% dimethyl sulfoxide (DMSO) and stored in the refrigerator until used.

Phytochemical Analysis

Freshly prepared extracts were subjected to standard phytochemical analysis to determine the presence of the following phytoconstituents, i.e., alkaloids, phenols, flavonoids, glycosides, tannins, saponins, steroids, terpenoids, sugar, and proteins (Edeoga and Okwu, 2005 and Egwaikhide and Gimba, 2007) [13, 14].

Test Microorganisms

The test microorganisms used were Gram-positive bacterial strains, i.e., *Bacillus subtilis* (MTCC No. 441), *Staphylococcus aureus* (MTCC No. 87), and *Staphylococcus hominis* (MTCC No. 8980) and Gram-negative strains were *Escherichia coli* (MTCC No. 40) and *Proteus vulgaris* (MTCC No. 742). These strains were procured from MTCC, Chandigarh. The bacterial species were first revived and then sub cultured in nutrient broth and incubated at 37°C for 24 h.

Antibacterial Activity

The antibacterial activity of the leaf extracts of *T. cordifolia* was determined by agar well diffusion method. The bacterial cultures used for testing were *E. coli*, *B. subtilis*, *S. aureus*, *S. hominis*, and *P. vulgaris*. The bacterial species to be evaluated for antibacterial activity were inoculated and maintained in nutrient broth for 24 h before use. Then, the culture was spread on nutrient agar media in Petri plates. Sterile Cork borer was used to bore wells into the agar. Approximately 50 µl of leaf extracts (1 mg/ ml) of different solvents (methanol, chloroform, ethyl acetate, diethyl ether, and aqueous) were introduced into the wells separately and streptomycin (1 mg/ml) as positive control and 10% DMSO as negative control were used. The plates were allowed to stand at room temperature for some time and then incubated at 37°C. These were observed for zones of inhibition after 24 h and compared with positive and negative control.

Antioxidant Activity

The antioxidant activity of leaf extracts of *T. cordifolia* was assayed by 1, 1–diphenyl-2-picryl hydrazyl (DPPH) scavenging method. The free radical scavenging activity of leaf extract was assayed using a stable free radical, DPPH. This DPPH method used here is a modification of Moon and Terao, (1998) [15] 300 µl of extract mixed with 2 µl of DPPH reagent (0.1 mM in methanol). The mixture was shaken vigorously and left to stand for 30 min. The absorbance of the resulting solution was measured at 517 nm in a spectrophotometer against the blank containing water instead of extract.

L-ascorbic acid was used as positive control. The experiment was repeated twice, and the percentage of DPPH scavenging was calculated using the following formula:

% scavenging = $B_{0\text{-}B1}/B_0 \times 100$

B_0-Absorbance without sample extract

B_1-Absorbance of sample extract with DPPH solution.

The decrease in the absorbance of DPPH solution indicates an increase in DPPH radical scavenging activity. Total antioxidant activity (TAA %) was expressed as the percentage inhibition of DPPH radical.

Results and Discussion

Phytochemical Analysis of Leaf Extracts in Various Solvents

Preliminary phytochemical screening of the leaf extract of *T. cordifolia* showed the presence of the various phytochemicals such as alkaloids, glycosides, flavonoids, steroids, tannins, terpenoids, saponins, and sugars in different solvent extracts (Table 1). Five different solvents (methanol, chloroform, ethyl acetate, diethyl ether, and aqueous) were used to obtain extracts of leaves and used for qualitative phytochemical analysis of leaves using standard chemical tests. The methanolic extract showed the presence of most of the phytochemical components.

Preliminary screening of phytochemicals is a valuable step, in the detection of the bioactive principles present in medicinal plants and subsequently, may lead to drug discovery and development. From the above results, it can be noted that successful extraction of biologically active compounds from plant are largely dependent on the type of solvent used during extraction. In this study, different solvents such as methanol, ethyl acetate, diethyl ether, chloroform, and aqueous were used. This study, therefore, validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract. [20] The methanolic extract contains most of the phytochemical components due to the high solubility of active compound of *T. cordifolia* in this solvent as compared to other solvents. Qualitative screening of *T. cordifolia* showed that the leaves extract of plant has alkaloids, flavonoids, phenols, tannins, steroids, and terpenoids. This variation and availability of phytochemicals makes this plants potential medicinal plant (Yamaguchi *et al.* 1998) [16]. Steroids were present in all solvent extracts. Steroids are responsible for cholesterol reducing properties and also help in regulating the immune response (Chen, 1997) [17]. Phenols and tannin were also present in all extracts except diethyl ether. Plants rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms (Joseph *et al.* 1999) [18].

Table 1: Results of the phytochemical screening tests of leaf extracts of *T. cordifolia* in different solvents

Type of solvent used for extraction	Test for the presence of phytoconsituents									
	Alkaloids	**Glycosides**	**Flavonoids**	**Phenols**	**Steroids**	**Tannins**	**Terpenoids**	**Saponins**	**Sugars**	**Proteins**
Chloroform	+	-	+	+	+	+	+	+	+	-
Methanol	+	+	+	+	+	+	+	-	+	-
Ethyl acetate	+	-	-	+	+	+	+	+	+	-
Diethyl ether	+	-	+	-	+	-	+	+	+	-
Aqueous	+	-	+	+	+	+	+	-	-	-

Antibacterial Activity of Leaf Extract of *T. cordifolia* in Different Solvents

Antibacterial activity of *T. cordifolia* was recorded against *E. coli*, *S. aureus*, *P. vulgaris*, *B. subtilis*, and *S. hominis*. The activity was measured in terms of zones of inhibition in diameter (mm) for methanolic, ethyl acetate, diethyl ether,

chloroform, and aqueous extracts of leaves tissue (Figure 1 and Table 2). The results revealed that the chloroform extract exhibit the effective antibacterial activity against the tested bacterial species except for *E. coli* and *B. subtilis*. It shows the maximum zone of inhibition for *P. vulgaris* and *S. aureus* as shown in Table 2 and Figure 1.

Table 2: Results of antibacterial activity of different solvent extracts against different strains of bacteria

Bacterial species	Zone of inhibition (mm) Test samples					
	Positive control (streptomycin)	Methanolic extract (Meth.)	Chloroform extract (Chl.)	EA	DEE	Aqueous extract (W)
E. coli	28	-	-	-	-	-
P. vulgaris	38	15.0	14.0	13.0	13.0	12.0
S. aureus	36	7.5	18.0	14.0	13.0	-
B. subtilis	32	-	-	-	-	-
S. hominis	20	12.0	5.0	7.0	-	15.0

EA: Ethyl acetate, DEE: Diethyl ether, *E. coli: Escherichia coli, S. aureus: Staphylococcus aureus, B. subtilis: Bacillus subtilis, S. hominis: Staphylococcus hominis, P. vulgaris: Proteus vulgaris*

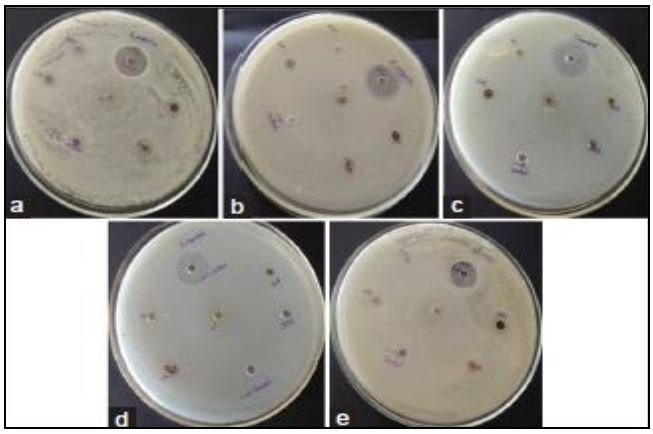

Fig 1: Antibacterial activity of different solvent extracts of leaf tissue of *Tinospora cordifolia* against (a) *Bacillus subtilis* (b) *Staphylococcus aureus* (c) *Staphylococcus hominis* (d) *Proteus vulgaris* (e) *Escherichia coli.* +ve control: Positive control (streptomyces = 1 mg/ml), -ve control: Negative control (1% DMSO), Chl.: Chloroform extract, Meth. Methanolic extract, EA: Ethyl acetate extract, DEE: Diethyl ether extract, and W: Aqueous extract

The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents. The plant contains numerous biologically active constituents many of which have antimicrobial properties. The antimicrobial activity of this plant was reported in root, stem and leaves extracts on pathogenic microorganisms (Mahesh and Satish, 2008; Smy, 2005) [19-20]. Antibacterial activity of tested plant extracts in the present study indicates the presence of bioactive agents. The results revealed that the methanolic, ethyl acetate and chloroform extract exhibit the effective antibacterial activity against the tested bacterial species except for *E. coli* and *B. subtilis*. *E. coli* and *B. subtilis* were not inhibited by the leaf extracts of *T. cordifolia*. However, chloroform extract shows the maximum size of the zone of inhibition than methanol and ethyl acetate extract, as shown in Table 2. It can be interpreted that the antibacterial activity against microorganisms is due to any one or more alkaloids of the plant (Nayak and Singhari, 2003) [24]. This finding supports the use of *T. cordifolia* in traditional system for its claimed uses for curing urinary and skin diseases, inflammation and fever.

Antioxidant Activity Test

The plant extracts were screened for their potential antioxidant traits. Antioxidant activity of leaf extract of *T. cordifolia* was measured by DPPH scavenging method. DPPH, a protonated radical, has characteristic absorbance maxima at 517 nm which decreases with the scavenging of the proton radical (Yamaguchi *et al.* 1998) [16]. Hydrogen donating ability of antioxidant molecule contributes to its free radical scavenging nature (Chen, 1997) [17].

In our results, leaves of *T. cordifolia* exhibited the good antioxidant activity in methanol and aqueous extracts; the methanolic extract has the higher antioxidant activity than aqueous extract.

Flavonoids and phenolic compounds in various plants have been reported to have multiple biological effects such as antioxidant, free radical scavenging abilities, anti-inflammatory, and anticarcinogenic properties (Thamaraiselvi, 2012) [21]. The presence of phenols, flavonoids, and tannins in methanolic and aqueous leaf extracts of *T. cordifolia* attributes this free radical scavenging activity. The free radical scavenging activity was less than the ascorbic acid which was taken as control. The TAA % value of methanolic extract, ethyl extract, chloroform extract, diethyl ether, and aqueous extract was 44%, 32%, 26%, 25%, and 14% and of control (ascorbic acid) was 72%. Thus, antioxidant rich leaf extracts of *T. cordifolia* serve as a source of nutraceuticals that alleviate the oxidative stress and helps in prevention and reduction of the degenerative diseases with consequent health benefits (Joseph *et al.* 1999 and Kitts *et al*. 2000) [18,22].

Therefore, extracts from these plants in different solvents could be seen as a good source of rich bioactive compounds. Thus, there is an urgent need to try as much as solvents as possible in qualitative phytochemical screening of plants (Dasgupta *et al.* 2013) [23].

Conclusion

T. cordifolia exhibited the potential antibacterial and antioxidant activities. The leaf extracts of this plant have various phytochemicals such as alkaloids, glycosides, flavonoids, steroids, tannins, terpenoids, saponins, and sugars which are responsible for these activities. The methanolic, chloroform and ethyl acetate extract have good antibacterial activity against the tested bacteria. The plant exhibited the admirable antioxidant activity in methanol and aqueous extracts. The observed good antioxidant activity of the extract indicates the potential of the leaves as a source of natural antioxidant.

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