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Comparative estimtion of antimicrobial and antioxidant activity of leaves and stems tissues of medicinal plant-*Tinospora cordifolia*

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Abstract
Tinospora cordifolia (Menispermaceae) is a divine herb due to its therapeutic efficacy. The main objective of the present study was to make a comparative evaluation of leaf and stem tissues of this medicinal plant for the presence of phytoconsituents and antibacterial &antioxidant activities. Different solvents (methanol, ethyl acetate, diethyl ether, aqueous and chloroform) extracts were analyzed for presence of phytochemicals and studied further for antimicrobial and antioxidant activities. The antibacterial and antioxidant activity was evaluated by Agar well diffusion & DPPH scavenging method.
Methanol proved to be best solvent when equated with other solvents (ethyl acetate, diethyl ether, chloroform and water) due to better solubility of metabolites in it. The stem tissue extracts showed the presence of more phytoconstituents and better antibacterial & antioxidant activities as compared to leaves. For the antibacterial activity, among the different solvent extract, the chloroform extract of stem tissue showed complete inhibition of all tested bacterial strains with maximum zone of inhibition. For antioxidant activity, the TAA % value of leaf tissue in methanol, ethyl acetate, chloroform, diethyl ether and aqueous extracts was 44%, 32%, 26%, 25%, and 14% and respectively of stem tissue was 70.70%, 67.00%, 62.00%, 59.10% and 69.70%. The Ascorbic acid was used as control. The TAA % value of ascorbic acid was 72%. The DPPH scavenging method showed that the antioxidant activity of stem extract in all solvents was superior as compared to leaf extract.
This investigation reveals that the stem part of *T. cordifolia* contains higher quantity of phytochemicals, due to which it showed better antibacterial and antioxidant activity than leaf tissue and thus has higher prospective applications in food, agriculture and pharmaceutical industry.

Keywords: *Tinospora cordifolia*, solvent extracts, phytochemicals, anti-bacterial and anti-oxidant

1. Introduction
The plant kingdom is a wealth house of prospective drugs and in the recent years there has been an increasing alertness about the prominence of medicinal plants. Plant derived medicines have been used for traditional health care in most parts of the world for thousands of years [1]. India is one of the richest countries in the world when we talk of diversity of medicinal plants [2]. The plants which have been chosen for medicinal purpose over thousands of years constitute the most evident choice of investigating the current search for therapeutically effective new drugs such as antiviraldrugs [3], antimicrobial drugs [4] and anti-hepatotoxic compounds. Also according to World Health Organization (WHO), for exploring variety of other drugs, the plants would be the best source. However; plants should be first investigated for better understanding of their medicinal properties, safety, and efficiency [5].
Tinospora cordifolia (*T. cordifolia*) which is genetically diverse, large, deciduous climbing shrub belonging to the family Menispermaceae [6-8] also known as Giloe, is an important medicinal plant used in Ayurvedic system of medicine. This plant found throughout India, especially tropical parts of country and also in china [9]. The mature stem is reported to cure intermittent and chronic fevers, jaundice, diabetes, respiratory illness, neurological disorders, rheumatism, and skin ailments [10-12]. Anti-inflammatory, anti-allergic, hypotensive, hepatoprotective, immunostimulant, diuretic [13-15] and anti- microbial properties of this plant have been clinically proved [16, 17]. The anti-neoplastic activity of this plant is also reported recently [18]. According to Patanjali Yogapith this plant is very effective in preventing epidemic swine flu and dengue.
Medicinal plants show medicinal properties due to presence of bioactive constituents [19-22] which provide definite physiological action on the human body. These can be derived from barks, leaves, flowers, roots, fruits and seeds [23]. These secondary metabolites were chemically

and taxonomically diverse compounds with obscure function. They are widely used in the human therapy, agriculture, scientific research and countless other areas [24].

The phytochemical analysis, antimicrobial and antioxidant activities of medicinal plants draw attention of plant researchers for standarazation of protocols for isolation and purification of these secondary metabolites and use them for pharmacological purposes. Phytoconstituents were investigated and found that these have various medicinal properties. Experimentally alkaloids proved their anti-diabetic, anti-protozoal and anti-inflammatory properties [25]. Flavonoids and phenolic compounds have been reported to have antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic properties [26]. Steroids are known for their insecticidal, analgesic properties, cardio tonic, central nervous system activities, immuno-modulatory properties [27, 28] antimicrobial and anti-inflammatory actions [26]. Tannins exhibit toxicity against bacteria and fungi [29].Terpenoids can be used as protective substances in storing agriculture products due to insecticidal properties [30].

The selection of plant extracts and natural products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents. It can be interpreted that the antibacterial activity against microorganisms is due to any one or more alkaloids of the plants [31]. The use of current microbiological techniques demonstrates that medicinal plants normally exhibit significant strength against human bacterial and fungal pathogens [32-34].

T. cordifolia is a gifted source of antioxidant agent. This plant is used in traditional medicine as an alternative source for the treatment of degenerative diseases triggered by free radicals. Natural antioxidants like phenolic compounds, flavonoids which are secondary metabolites present in food products of plant origin [35, 36] can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [37]. These also exhibit a wide range of biological effects like anti-ageing, anti-mutagenicity and show their protective effects on oxidative stress [38-40]. Several studies have shown that plant derived antioxidant nutraceuticals scavenge free radicals and modulate oxidative stress related degenerative diseases [41].

Determination of biologically active metabolites from plant material is highly dependent on the type of solvent used for the extraction [42]. This emphasizes the need to try large number of solvents for screening plant parts for secondary metabolites. In the present study, various solvents i.e. water, methanol, chloroform, diethyl ether and ethyl acetate were used to acquire extracts of *Tinospora cordifolia* leaves and stems. These extracts were used further for preliminary phytochemical analysis by standard chemical methods [43]. Subsequently, the stems and leaves extracts were compared and checked for the presence of better quantity of phytoconstituents as these phytoconstituents were responsible for superior antibacterial and antioxidant activities. And hence the plant part which shows best results can be preferred further for medicinal and other related purposes.

2. Materials and Methods

2.1 Collection of Plant Material

The leaves and stems of the medicinal plant- *Tinospora cordifolia* were collected from Botanical Garden of Kurukshetra University, Kurukshetra, Haryana, India. The samples were brought to the laboratory in sealed plastic bag and stored at 4 °C. The material was identified by Prof. B. D. Vashistha of Department of Botany, Kurukshetra University, Kurukshetra. A voucher specimen (Herbarium/ Bot. K.U./Biotech.-3-2017) of the plant was deposited at herbarium, Department of Botany, Kurukshetra University Kurukshetra.

2.2 Grinding of plant materials

The plant material (Leaves and Stems) were taken, washed under running tap water followed by washing twice with distilled water. The plant material was shade dried before grinding. After drying, plant material was grounded to powdered form. The powdered plant material was used further for extraction procedure.

2.3 Extract Preparation

Coarsely powdered plant material was extracted for 8 hours with different solvents (diethyl ether, ethyl acetate, chloroform, methanol and water) in Soxhlet apparatus. The extracts were filtered and allowed to evaporate. The dried extracts were dissolved in 10% DMSO (dimethylsulfoxide) and stored in refrigerator until used.

2.4 Phytochemical Analysis

Freshly prepared extracts were subjected to standard phytochemical analysis to determine the presence of the following phytoconstituents i.e. alkaloids, tannins, phenols, terpenoids, sugar, flavonoids, glycosides, saponins, steroids and proteins [44, 45].

2.5 Antibacterial Activity

The antibacterial activity of the leaf and stem extracts of *T. cordifolia* was determined by agar-well diffusion method. The bacterial cultures used for testing were *Bacillus subtilis* (MTCC No. 441), *Staphylococcus aureus* (MTCC No. 87), *and Staphylococcus hominis* (MTCC No. 8980), *Escherichia coli* (MTCC No. 40) *and Proteus vulgaris* (MTCC No. 742). The cultures were procured from IMTECH, Chandigarh. The bacterial species were first revived and then sub-cultured in nutrient broth and incubated at 37 °C for 24 hr.

2.6 Antioxidant activity

The antioxidant activity of leaf and stem extracts of *T. cordifolia* was assayed by DPPH scavenging method. The free radical scavenging activity of leaf and stem extract were assayed using a stable free radical, 1, 1–diphenyl-2-picryl hydrazyl (DPPH). This DPPH method used here is a modification of Moon and Terao [46]. The percentage of DPPH scavenging was calculated using the following formula:

$$\% \text{ Scavenging} = B_0 - B_1/B_0 \times 100$$

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.

3. Results and Discussion

3.1 Phytochemical analysis of leaf and Stem extracts of different solvents

The preliminary analysis of leaf and stem portion of *T. cordifolia* revealed the presence of different medicinally important phytocompounds. Different solvents of differ

polarities helped in extraction of different phytochemicals from the plant. Variations of solvent types used do affect the presence of bioactive compounds of an extract [47-49]. The choice of solvents is made by considering different factors such as class of phytochemicals, diversity and polarity of compounds to be extracted [50].

The presence of variety of phytochemicals in *T. cordifolia* i.e. tannins, flavonoids, phenols, terpenoids and glycosides in extracts of methanol, diethyl ether, isoamyl alcohol, water, butanol and ethyl acetate was also detected by Paul *et al.* in 2016 [51].

In our study, five different solvents viz. methanol, ethyl acetate, chloroform, diethyl ether and aqueous were used to extract phytoconstituents from leaf and stem tissues of *T. cordifolia.* Qualitative phytochemical analysis was done using standard chemical methods. The results were analyzed for the presence of alkaloids, saponins, steroids, sugars, phenols, glycosides, tannins and proteins (Table 1). Stem and leaf extracts of *T. cordifolia* revealed different results. In comparison to leaf, stem extract showed better results for the presence of phytochemical components. Among the different solvent used methanol, ethyl acetate and water extract of both (leaf and stem) parts showed the presence of higher phytochemicals than diethyl ether and chloroform extracts. Methanol was the best solvent when compared with ethyl acetate and water because of the presence of maximum phytocompounds in its extract. Alkaloids, steroids and terpenoids were present in all solvent extracts of leaf and stem. Alkaloids are naturally occurring chemical compounds having pharmacological properties and used directly for medications [52]. The steroids plays important role in regulating immune response and also responsible for cholesterol reducing properties [53]. Phenols and tannin were also detected in all extracts except diethyl ether extracts of both leaves and stems. Phenolic compounds are responsible for multiple biological effects like antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic properties [54]. Tannin and phenols present in medicinal plants possess antimicrobial activities against a number of microorganisms [55, 56-58]. These secondary metabolites also contributes significantly towards other biological activities of the plants such as anti-malarial, anti-inflammatory, anti-carcinogenic and anti-oxidant etc. [59, 60]. This variation and availability of phytochemicals inside plant parts makes this plant an important medicinal plant. The screening of plant extracts for these phyto-constituents helped further to analysis antimicrobial and antioxidant activities of stem and leaf parts of *T. cordifolia.*

3.2 Antimicrobial activity of different plant parts (leaves and stems) of *Tinospora cordifolia* in different solvents:

Different solvent (aqueous, ethanol and chloroform) extracts of stem tissues of *T. cordifolia* against *E. coli, P. vulgaris, E. faecalis, S. typhii, S. aureus* and *S. marcescenses* was studied by Jeychandran *et al.* (2003) and found that the ethanolic extract has significant antibacterial activity [56]. In our study, we have tried five different solvent extracts of leaf and stem portion of *T. cordifolia* and tested them against *E. coli, P. vulgaris, S. aureus, S. hominis* and *B. subtilis*. The stem extracts showed the higher antibacterial activity in form of zones (mm) (Figures 1, and Table 2) as compared to leaf against the bacterial species used. The antibacterial activity of leaf extracts of diverse solvents was presented in our previous study [61]**.** All solvent extracts of stem tissues showed significant antibacterial activity but chloroform extract display complete inhibition of all used bacterial strains with maximum zone of inhibition followed by diethyl ether extract. Diethyl ether extract proved somewhat better as compared to water, methanol and ethyl acetate extracts. The *E. coli* and *B. subtilis* strains were inhibited by all solvents extracts of stem tissues but not subdued by leaf extracts [58]. Similar conclusion, that different solvents extracts of stem tissues is better than leaf extracts of *T. cordifolia* was presented by Shanthi *et al.*(2013) [62]. The antibacterial activity was screened to authenticate the use of *T. cordifolia* against bacterial infections.

Table 1: Phytochemical Analysis of different plant parts (Leaves and Stems) of *Tinopora cordifolia* in different solvents:

Samples	**Leaf Extract**					**Stem Extract**				
Samples / Tests for	**M**	**W**	**EA**	**DEE**	**Chl.**	**M**	**W**	**EA**	**DEE**	**Chl.**
Alkaloids	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	-	+	-	+	+	+	-	+
Glycosides	+	-	-	-	-	+	+	+	+	+
Phenols	+	+	+	-	+	+	+	+	-	-
Steroids	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	-	+	+	-	+	-	+
Terpenoids	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	+	+	+	+	+	-	+	+
Sugars	+	-	+	+	+	+	+	+	+	+
Proteins	-	-	-	-	-	+	+	+	+	+

(Meth. - Methanolic Extract, W- Aqueous extract, Chl. - Chloroform Extract, EA- Ethyl Acetate Extract, DEE- Diethyl ether Extract)
(+ means presence and – means absent of phytoconstituents)

Table 2: Antimicrobial activity of different plant's parts (Leaves and stems) of *Tinospora cordifolia* in different solvents.

Bacterial strains	**Leaf**						**Stem**					
	+ve control	**Meth.**	**W**	**Chl.**	**EA**	**DEE**	**+ve control**	**Meth.**	**W**	**Chl.**	**EA**	**DEE**
E. coli	28	-	-	-	-	-	34	-	-	17	-	13
P. vulgaris	38	15	12	14	13	13	38	15	13	14	-	12
S. aureus	36	7.5	-	18	14	13	36	15	-	11	10	15
S. hominis	20	12	15	5.0	7.0	-	32	10	13	15	11	15
B. subtilis	32	-	-	-	-	-	32	-	-	12	-	-

[+ve control - Positive control (Streptomycin - 1mg/ml), -ve control - Negative control (1% DMSO), Meth. - Methanolic Extract, W- Aqueous extract, Chl. - Chloroform Extract, EA- Ethyl Acetate Extract, DEE- Diethylether Extract]

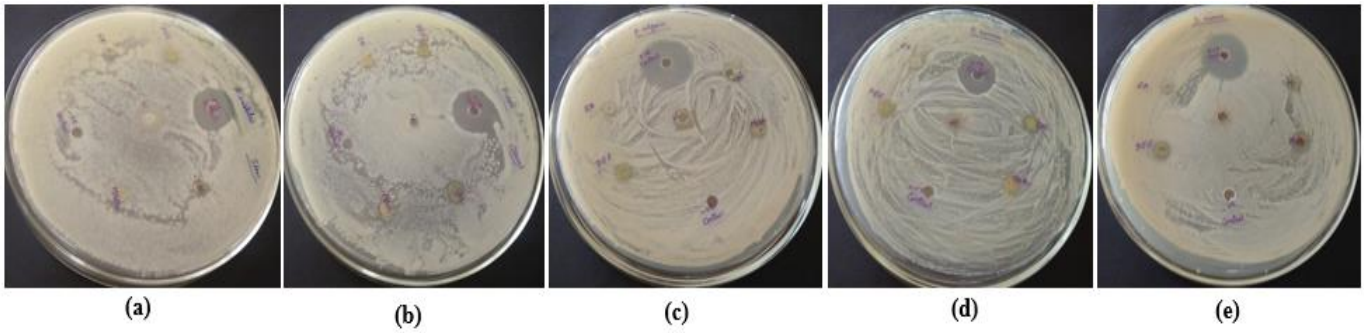

Fig 1: Antibacterial activity of different solvent (Methanol, Chloroform, Ethyl Acetate, Diethylether and Aqueous) extracts of stem tissue of *T. cordifolia* against (a) *B. subtilis (*b) *E. coli* (c) *P. vulgaris* (d) *S. hominis (*e) *S. aureus*
[+ve control =Positive control (Streptomycin = 1mg/ml), -ve control = Negative control (1% DMSO), Chl. = Chloroform Extract, Meth. = Methanolic Extract, EA= Ethyl Acetate Extract, DEE= Diethylether Extract, W= Aqueous extract]

3.3 Antioxidant activity of different plant parts (Leaf and stem) of *Tinospora cordifolia* in different solvents:

The presence of phenols, flavonoids and tannins in solvent extracts of leaf and stem part of *T. cordifolia* attributes free radical scavenging activity. Most of phytochemical compounds have antioxidant activity, protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. The free radical scavenging activity was less than the ascorbic acid which was taken as control. The TAA % value of leaf tissue studied in methanol, ethyl acetate, chloroform, diethyl ether and aqueous extracts were 44%, 32%, 26%, 25%, and 14% which was discussed in our previous work[58] and the TAA% of stem tissue in methanol, ethyl acetate, chloroform, diethyl ether and aqueous extracts were 70.70%, 67.00%, 62.00%, 59.10% and 69.70% & of control (Ascorbic acid) was 72% as shown in figure 2.

So, as per our results, stem part of *T. cordifolia* exhibited good antioxidant activity then its counterpart leaf tissue. And among the different solvents tried the methanol extract showed the highest antioxidant activity followed by ethyl acetate, chloroform, diethyl ether and aqueous extracts. Thus, antioxidant rich leaf and stem extracts of *T. cordifolia* may serve as source of nutraceuticals which further helps in prevention and reduction of the degenerative diseases with consequent health benefits [55, 63]. In comparison with leaf tissue, the stem tissue showed the better antioxidant activity with regard to different parameters analyzed. Thus, stem portion of *Tinospora cordifolia* can be considered as a more potent source of natural antioxidant over leaf tissue.

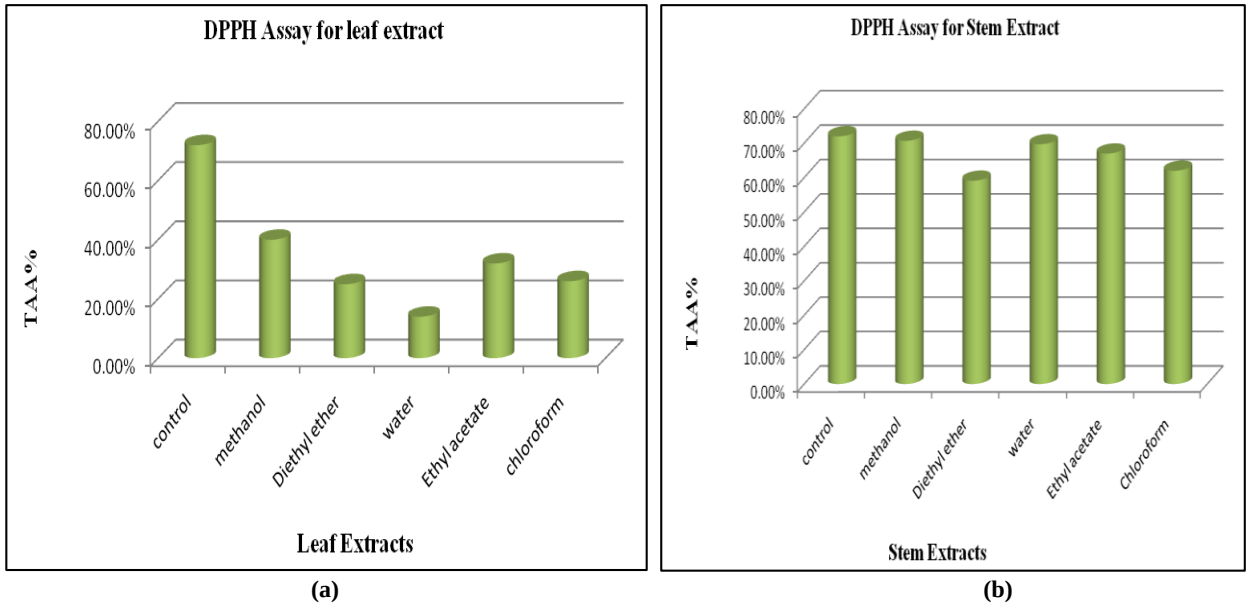

Fig 2: Antioxidant activity of *T. cordifolia* in different solvents (Methanol, Diethyl ether, water, ethyl acetate and chloroform) (a) Leaf Extracts (b) Stem Extracts.

4. Conclusion

The results of the following study indicated that *Tinospora cordifolia* is a rich source of bioactive metabolites and types of solvents used do affect the presence of bioactive compounds of an extract. Different plant parts may have unlike amount and types of metabolites as stems parts displayed better antibacterial and antioxidant activities as compared to leaves tissues. This study may be useful in developing new medicines & specialized drugs with more efficiency.

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