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In Vitro Regeneration and Comparative Analysis of Antimicrobial and Antioxidant Activity of Wild and Micropropagated *Tinospora cordifolia*

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Abstract

Tinospora cordifolia is widely used in Ayurvedic system of medicine. The main objective of our present study was to do *in vitro* propagation and to evaluate bioactive metabolites, anti-bacterial and antioxidant activity of wild and *in vitro* extracts of *T. cordifolia*. *In vitro* regeneration of *T. cordifolia* was tried using different explants placed on MS media with different concentrations and combinations of cytokinins and auxins. Nodal explants were selected for regeneration due to its superior regeneration potential than other explants. Kinetin (3mg/l) was good for shoot regeneration and elongation than BAP. The 2, 4-D along with BAP (1.5+ 2.0 mg/l) exhibited good callus production. IAA (1mg/l) displayed better results for root induction than IBA. Different solvent (methanol, ethyl acetate, Diethyl ether, aqueous and chloroform) extracts of wild and *in vitro* regenerated plant were analyzed for existence of phytochemicals and studied further for evaluation of antibacterial and antioxidant activities. The antibacterial and antioxidant activity was evaluated by Agar well diffusion & DPPH scavenging method. Alkaloids, steroids, phenols, tannins, flavonoids, sugar, terpenoids, proteins and carbohydrates were presented in wild plant extracts while callus tissue and *in vitro* regenerated plant extracts showed the presence of only alkaloids, phenol, terpenoids, steroids and flavonoids while saponins, glycosides and tannins were found to be absent. The antibacterial activity of wild plant was somewhat superior to callus and *in vitro* regenerated plant. The methanol extract exhibited good antibacterial activity in all sample (wild, callus and *in vitro* plant) extracts. The antioxidant activity of the wild plant, callus tissue and *in vitro* regenerated plant extracts in diverse solvents was evaluated. The TAA% value of wild plant, callus tissue and *in vitro* regenerated plant in methanol, ethyl acetate, chloroform, Diethyl ether & water solvent extracts were (89.26%, 83.27%, 76.28%, 60.67% and 81.11%), (48.45%, 51.52%, 40.78%, 32.00% and 35.27%) and (33.78%, 24.67%, 12.52%, 11.22% and 20.24%) and of Ascorbic acid (control) was 94.96%. The methanol extract of wild plant and *in vitro* regenerated plant and ethyl acetate extract of callus tissue showed maximum inhibition (89.26%, 33.78% and 51.52%). This investigation reveals that the extracts of wild and *in vitro* regenerated *T. cordifolia* are

potential source of metabolites which are responsible for good antibacterial and antioxidant activities.

Keywords
Tinospora cordifolia, Plant growth regulators, Callus, Phytochemicals, DPPH, Ascorbic acid.

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1. INTRODUCTION

Medicinal plants are an important source of medicines and used worldwide due to their contribution to healthcare and wellbeing of human societies. The plant-based medicines have less toxicity, and their importance is being realized in both developed and developing countries [1]. Today's health care systems rely largely on plant material. Diverse parts of the plant such as roots, stem, flowers, fruits, leaves, seeds and the whole plants are used in a variety of forms of medicines. Medicinal plants were found to be sources of various phytochemicals that could be used for the production of pharmaceuticals, as additives in cosmetic, food or drink supplements [2]. The medicines derived from the plants have been used for traditional health care in the largest part of the world for thousands of years [3]. According to World Health Organization (WHO), plants would be the best source for exploring variety of other drugs.

Tinospora cordifolia (Willd) Miers (Synonym: *T. sinensis* (Lour.) Merr.) belongs to the family Menispermaceae, is an Indian medicinal plant. Throughout the centuries, this plant has been used for the treatment of a range of ailments in Ayurvedic preparation. Studies on medicinal importance of *T. cordifolia* report anti-inflammatory, antiarthritic [4], antioxidant [5], osteoprotective [6], antidiabetic [7], anti-pyretic [8, 9], anti-infective [10], antiallergic [5], immunomodulatory [11], hepatoprotective [12], cardioprotective activities [13], anti-malarial and anti-hyperglycemic activities [14]. These medicinal properties are due to presence of a variety of bioactive components like steroids, diterpenoid lactones, saponins, flavonoids, terpenoids, glycosides and alkaloids etc. isolated from the different parts of the plant.

But now days, *T. cordifolia* is on the verge of extinction because of its broad applications in conventional medicines and also in current medication. Therefore, we need an alternative method for enhancing the concentration of essential bioactive metabolites. The establishment of tissue culture holds great promise for any advance study. Micro propagation is a reliable method for mass multiplication of existing stocks of germplasm for enhancement of biomass and also for the conservation of threatened species [15-21]. Tissue culture methodology has shown promise for clonal multiplication of wild populations and economically important trees which have been cultivated for generations.

Phytocompounds were investigated and found that these have various therapeutic properties. The screening of plant extracts for antimicrobial activity has revealed that these medicinal plants signify a potential source of novel anti-infective agents. It can be inferred that the antibacterial activity against microbes is due to any one or more alkaloids of the plants [22]. The use of current microbiological techniques validates that medicinal plants normally unveiled significant strength against human bacterial and fungal pathogens [23-25].

Tinospora cordifolia is also a prospective source of antioxidant agent. Phytochemicals such as phenols and flavanoids present inside various medicinal plants have been reported for multiple biological effects like antioxidant, free radical scavenging activity and anticarcinogenic properties [26]. Free radicals are synthesized in our body due to biological oxidation. These free radicals when present in excess cause damage to the body and also contribute to oxidative stress [27,28]. The compounds that scavenge harmful free radicals have enormous potential to protect our body against this disease processes [29-31]. The information regarding the *in vitro* anti-oxidative potential of *T. cordifolia* is sparse Determination of bioactive compounds from medicinal plant is highly dependent on the type of solvent used for the extraction purpose [32]. This give emphasis to the need to try huge number of solvents for screening various plant parts for natural metabolites. The aim of the present research work was to evaluate phyto-constituents and to determine antioxidant activity of wild plant, callus tissue & *in vitro* regenerated *T. cordifolia* using different solvents i.e. methanol, ethyl acetate, chloroform and aqueous, so that, the results of the study can be further use for screening and characterization of bioactive metabolites of this plant and validate its use for therapeutic purposes.

2. METHODOLOGY

Plant sample and Tissue culture

Leaf, Stem and Root segments of wild *Tinospora cordifolia* plant (located in Botanical garden, Kurukshetra University, Kurukshetra, Haryana, India) were collected and stored at 4°C. The identifications and authentication of the plant was done 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.

For tissue culture studies, the plant material first washed several times under tap water followed by distilled water wash then surface sterilized under laminar air flow cabinet. The surface sterilized explants were cultured on MS medium supplemented with diverse concentrations and combinations of plant growth regulators (Auxins and Cytokinins) and maintained at 25±2°C for *in vitro* regeneration studies. The incubated explants were allowed to grow for a period of 4 weeks. The nodal portion exhibited good regeneration capability as compared to leaf and root explants. So, nodal explants were preferred for further regeneration studies.

After 4 to 6 weeks of culture, callus & shoots were established from stem explants. The callus formed was periodically sub-cultured for further multiplication.

The individual shoots were excised and cultured for rooting in MS medium containing IAA (Indole-3-acetic acid) and IBA (Indole-3-butyric acid) growth regulators. After differentiation and establishment of plantlets, the rooted plants were removed from culture vessels and transferred to plastic pots. The potted plants were maintained inside the culture room having optimum conditions such as 25±2°C temperature with a photoperiod of 16/8-hour (light/dark). Subsequently after 25-30 days, the plantlets were transferred to other pots containing normal garden soil and kept beneath shade in green house for another 2 weeks before transferring to open field.

The *in vitro* regenerated plantlets and the proliferated callus tissue from the stem explants were used for phytochemical estimation and compared with their wild counterpart.

Preparation of plant extracts using different solvents

The fresh plant material were taken inside lab and washed appropriately under tap water to remove soil and other contaminants followed by rinsing with distilled water. The sample was dried at 37°C for 2-3days. After drying, it was grounded to powdered form followed by extraction using different solvents (10g/100ml) i.e. ethyl acetate, chloroform, methanol, diethyl ether and aqueous by soxhlet apparatus. Extract were filtered and allowed to evaporate. The extract residues were used for qualitative analysis. The *in vitro* propagated plants and callus tissue were dried and extracted using same methodology.

Phytochemical Analysis

The phytochemical analysis was done to detect the presence of bioactive metabolites inside the plant extracts. Various phytochemical methods were used to check the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, glycosides, terpenoids, phenols, proteins and steroids by observing the change in color or formation of precipitate after addition of specific reagents [33, 34].

Antibacterial Activity

The antibacterial activity of the wild, callus and *in vitro* regenerated plant extracts of *T. cordifolia* was determined via agar-well diffusion method. The bacterial cultures used for testing were *Bacillus subtilis* (MTCC No. 441), *Staphylococcus aureus* (MTCC no. 87), *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 revived and then sub cultured on nutrient broth followed by incubation at 37°C for 24 hr. 50 µl of sample extracts (1mg/ml) of different solvents (Methanol, chloroform, Ethyl acetate, Diethyl ether and aqueous) were introduced into the wells separately. Streptomycin (1mg/ml) & 10% DMSO used as positive and negative control. The plates were incubated at 37°C. These were observed for zones of inhibition (cm) after 24 hrs and compared with positive and negative control.

Antioxidant Activity

The antioxidant activity of sample extracts of wild plant, callus tissue and *in vitro* regenerated plant was assayed by DPPH scavenging method. The free radical scavenging activity was checked by using a stable free radical, 1, 1–diphenyl-2-picryl hydrazyl (DPPH). This DPPH method used here is a modification of Moon and Terao [35], 300µl of extract mixed with 2 µl of DPPH reagent (0.1mM in methanol). The sample mixture was vigorously shaken and left for 30 minutes. The absorbance of the resulting solution was measured at a wavelength of 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 thrice, and the percentage of DPPH scavenging was calculated by this formula:

Scavenging % = $B_0 - B_1 / B_0 \times 100$

B_0 -Absorbance without sample extract

B_1-Absorbance of sample extract with DPPH solution

If there is a decrease in the absorbance of DPPH solution, it indicates an increase in DPPH radical scavenging activity. TAA% (Total antioxidant activity) was expressed as the % inhibition of DPPH radical.

3. RESULTS AND DISCUSSION

In Vitro Propagation

Tinospora cordifolia was regenerated through micro propagation by using different combinations and concentrations of phytohormones. The shoot induction via nodal explants exhibits superior results as compared to other explants i.e. internodes and leaf. Results of our study are in good agreement to the study by Sivakumar *et al.,* in which they established a procedure for rapid micro propagation of *Tinospora cordifolia* plant via node, internodes and shoot tips explants inoculated on MS medium supplemented with different concentration of growth regulators and observed that nodal tissue gave the best results as compared to other explants [36].

In our study, the data on effective *in vitro* regeneration of nodal explants in terms of shoot regeneration, rooting and callusing of *T. cordifolia* on standardized MS medium is presented in Table 1 and figure 1 and 2. Various concentrations and combinations of plant growth regulators for shooting, rooting and callusing for the plant *T. cordifolia* were tried & best concentrations were selected as shown in Table 1 and 2. Kinetin at concentration 3mg/l showed 100% shoots regeneration with two shoot from single explant with 2.86 cm shoot length, while BAP showed 95% shoot regeneration at same concentration. Thus, Kinetin was considered better for shoot regeneration and elongation than BAP growth regulator.

Table 1: Morphogenic response of different growth regulators for shooting and rooting of *T. cordifolia*

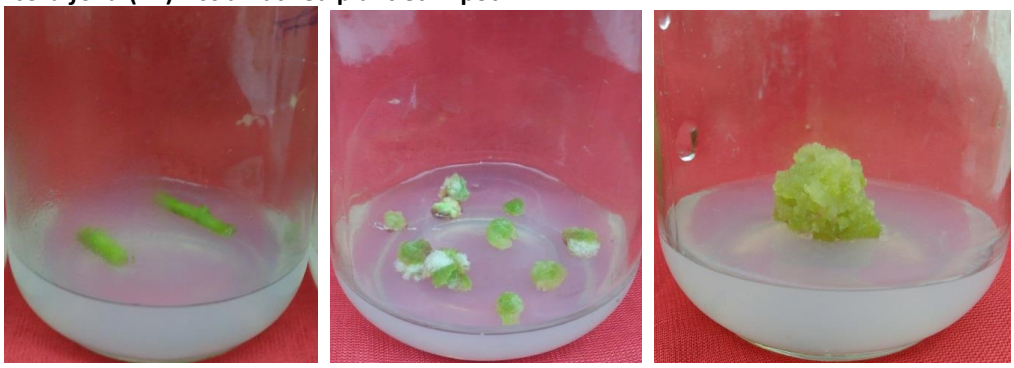
Type of Growth Regulators (Cytokinins)	Conc. (mg/l)	Shoot Development (%)	Shoot no./ Explant	Shoot Length (cm)
BAP	0.5	callus	-	-
	1.0	callus	-	-
	3.0	95	3.66±1.25	3.66±1.25
	5.0	84	3.98±1.40	3.98±1.40
Kinetin	0.50	60	2.12±0.00	1.82±0.05
	1.0	80	2.56±0.12	1.98±0.03
	3.0	100	2.76±0.16	2.86±0.07
	5.0	90	2.35±0.06	1.55±0.02
Type of Growth Regulators (Auxins)	**Conc. (mg/l)**	**Roots Development (%)**	**Mean no. of roots/shoot**	**Mean Root length (cm)**
IAA	0.5	70	3.7±0.18	2.9±0.6
	1.0	90	5.0±0.6	3.7±0.5
	2.0	60	2.0±0.3	2.5±0.7
IBA	0.5	80	3.5±0.2	1.8±0.6
	1.0	60	2.7±0.8	2.2±0.8
	2.0	40	1.6±0.21	1.5±0.1

Fig. (a) (b) (c) (d)

(e) (f) (g) (h)

(i) (j) (k)

Fig.1 (a-h): Various stages of regeneration process in nodal explants of *T. cordifolia:* (a) *Tinospora cordifolia*-medicinal plant (b) Nodal explants grown on MS media (c-e) Shoot bud initiation from nodal explants (f) Shoot differentiation and elongation (g) Roots formation on the excised shoot on MS media. (h) Rooted *Tinospora cordifolia* (i-k) Acclamatized plantlet in pot.



(a) (b) (c)

(d) (e)

Figure 2 (a) Nodal explants on MS medium containing BAP and 2, 4-D growth regulators (b-e) Stages of callus induction, proliferation and establishment from *T. cordifolia*.

Table 2. Effect of plant growth regulators on callus induction in *T. cordifolia*

Plant growth regulators (mg/l)		
BAP	**2,4-D**	**% Callus formation**
0.5	2.0	45
0.5	3.0	68
1.5	2.0	78
1.5	3.0	60

Callus regeneration was mainly used for indirect organogenesis. Better response of callus regeneration was observed from the nodal explants than leaf and inters nodes. In our study, when we used auxin 2, 4-D in combination with BAP (at lower concentration) for callus production then callus showed enhanced growth. 2, 4-D at concentration 2.0mg/l with BAP at concentration 1.5 mg/l showed the 78% callus regeneration and the concentration of 2, 4-D (1.5mg/l) with BAP (0.5mg/l) showed 68% callus regeneration from nodal explants on MS Media. The callus texture was friable and the colour of the callus was light green.

Different concentrations of auxins such as IAA and IBA were used for rooting and among them the highest degree of root induction was found on the media containing 1mg/l concentration of IAA, it displayed 90% of roots development within 25-28 days while the IBA at the same concentration presented 60% of roots formation (Table 1). Thus, we used IAA for rooting in *T. cordifolia*.

After establishment of plantlets, the rooted healthy plants were taken out and transferred to plastic pots filled with sterile course sand and garden soil in 1:1 ratio, moistened with autoclaved tap water. The potted plantlets were maintained inside the culture room at 25±2°C, with a photoperiod of 16/8-hour (light/dark). After 30 days, plantlets were transferred to pots which contain normal (non-autoclaved) garden soil and kept under shade in green house for 2 weeks before transferring to open fields.

Phytochemical analysis

The extraction and further characterization of bioactive metabolites from plants depends mainly upon the solvents used for extraction [37]. A broad chemical investigation has been done on *Tinospora cordifolia* and a large number of phytoconstituents belongs to diverse classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenols and aliphatic compounds has been isolated by Singh et al., 2003 [38]. In our study, the extracts of wild plant, callus tissue and *in vitro* raised plant in diverse solvents (methanol, chloroform, ethyl acetate, diethylether and aqueous) were prepared for qualitative phytochemical analysis using standard chemical tests and the results were compared. These extracts of the wild plant, callus tissue and *in vitro* regenerated *T. cordifolia* revealed the presence of the various phytochemicals i.e. alkaloids, glycosides, flavonoids, steroids, tannins, terpenoids, saponins and sugars (Table 3).

A preliminary phytochemical screening for qualitative determination of the phytoconstituents inside the ethanolic and hydromethanolic stem extracts of *T. cordifolia* was also reported by Mishra et al., 2014 [39]. In our study, among different solvents, methanol and ethyl acetate solvents were good extractor than water and chloroform because these solvents displayed maximum presence of phytocompounds. Among all solvents tried for extract preparation, methanol indicates the maximum presence of phytochemicals in all sample tissue extracts. Wild plant exhibited all phytochemicals but only flavonoids, alkaloids, phenols, sugars, terpenoids and steroids were present in callus tissue and *in vitro* regenerated plant extracts. Saponins, glycosides, proteins and tannins were completely absent. Among all solvent extracts, methanol extract of callus tissue showed the presence of tannins.

A noticeable decrease in the presence of phytochemicals was observed in *in vitro* propagated plants when compared to the wild plant (Table 3). Numerous reasons explain the differences in the concentration of phytoconstituents such as genetic diversity, environmental conditions, type of nutrients present inside soil or culture medium, climatic conditions and bioavailability of nutrients may alter the metabolic activities of medicinal plants [40]. Randriamampionona et al., (2007) also observed that there is lot of difference in phytoconstituents quantity among various samples taken from different locations [41]. The callus tissue showed maximum presence of phytoconstituents than *in vitro* raised plant. Callus is undifferentiated tissue mass and mainly utilized for somaclonal variations, these variations may be responsible for enhancement of phytochemical contents inside callus tissue than *in vitro* plant which was directly regenerated from nodal portion and grown inside open fields.

Table 3. Phytochemical analysis of wild plant, callus tissue and *in vitro* regenerated plant extracts

Samples	Wild plant					Callus tissue					*In vitro* regenerated plant				
Tests for	**Met**	**EA**	**Chl**	**W**	**DEE**	**Met**	**EA**	**Chl**	**W**	**DEE**	**Met**	**EA**	**Chl**	**W**	**DEE**
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Glycosides	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	-	+	+	-	+	-
Proteins	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
Sugars	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Tannins	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	-	+	-	+	+	-	+	+

Note: The symbols represented that, Met -Methanol, Chl -Chloroform, W –Aqueous, DEE- Diethyl ether and EA -Ethyl acetate and the symbol + means presence and – means absence of phytocompounds inside the samples.

The presence of phytocompounds inside *in vitro* raised *T. cordifolia* displayed the potential utility of tissue culture technique for further enhancement of these phytoconstituents at large scale. Therefore *in vitro* regenerated *T. cordifolia* can be used as an effective source of desired bioactive metabolites without destroying the wild plant.

Antibacterial Activity:

The presence of phytochemicals such as alkaloids, saponins and tannins in the plants play a major part in pharmaceutical field. Kubmarawa et al., (2007) and Mensah et al., (2008) also reported the significance of bioactive metabolites in various antibiotics, used in treating common pathogenic strains [42, 43]. The antibacterial activity of wild, callus tissue and *in vitro* regenerated plant extracts in various solvents (methanol, chloroform, ethyl acetate, diethyl ether and water) was evaluated by Agar well diffusion method. The wild plant extracts exhibited the maximum inhibition of microbial strains. Antibacterial activity of wild plant was found to be best when compared to callus and *in vitro* regenerated plant. Wild plant extracts inhibited all the bacterial strains (*B. subtilis*, *E. coli*, *S. aureus*, *S. hominis* and *P. vulgaris*) used for testing the activity. The callus tissue extracts inhibited *B. subtilis, S. aureus* and *S. hominis.* The bacterial strains *P. vulgaris* and *E. coli* was not inhibited by any solvent extracts of callus tissue. The bacterial sp. *S. aureus* and *S. hominis* were repressed by methanol and ethyl acetate solvent extracts of *in vitro* regenerated plant. The *in vitro* plant extracts have less capability to inhibit the tested bacterial strains than callus and wild plant.

The methanol extract exhibited good antibacterial activity in all sample (wild, callus and *in vitro* plant) extracts. It was a good solvent for extraction of maximum metabolites used for further biological activities. The callus tissue and *in vitro* regenerated plant extracts also indicated the antibacterial activity but less as compared to wild plant extracts (Table 4, fig. 3, 4 & 5). This proved that these *in vitro* plants retain their parental characteristics of phytochemical biosynthesis which were responsible for antimicrobial activity and can be enhanced further by altering the physical and hormonal parameters of cultural environment.

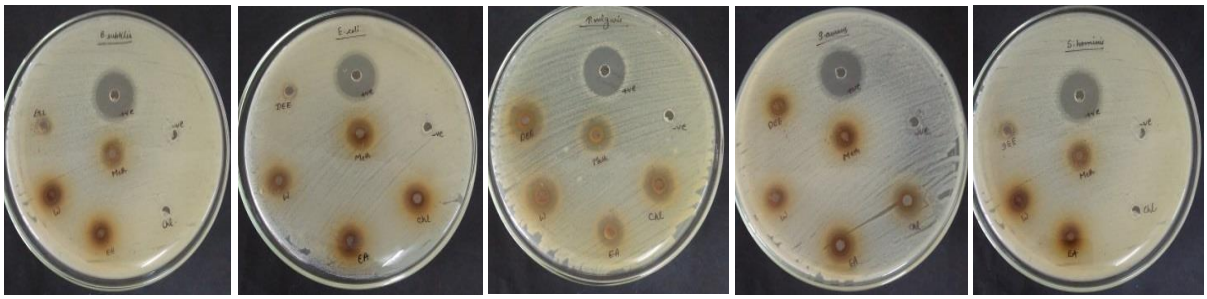


Figure 3: Antibacterial activity of different solvent (Methanol, Chloroform, Ethyl Acetate, Diethylether and Aqueous) extracts of wild *T. cordifolia* plant against (a) *B. subtilis* (b) *E. coli* (c) *P. vulgaris* (d) *S. aureus* (e) *S. hominis*

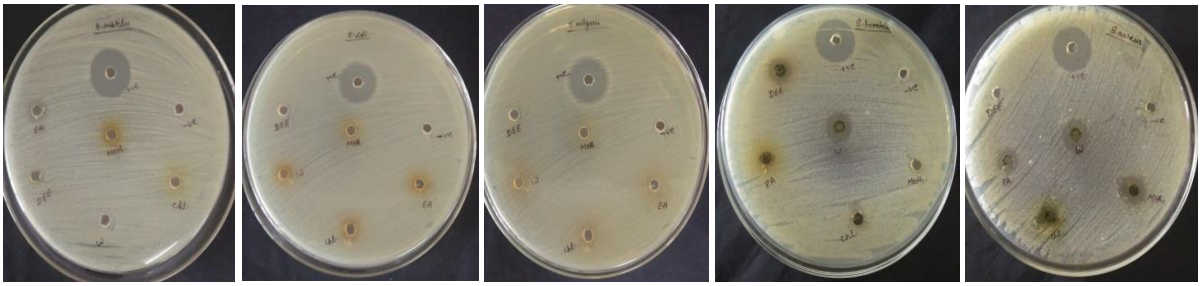


Figure 4: Antibacterial activity of different solvent (Methanol, Chloroform, Ethyl Acetate, Diethyl ether and Aqueous) extracts of callus tissue of *T. Cordifolia* plant against (a) *B. subtilis* (b) *E. coli* (c) *P. vulgaris* (d) *S. aureus* (e) *S. hominis*

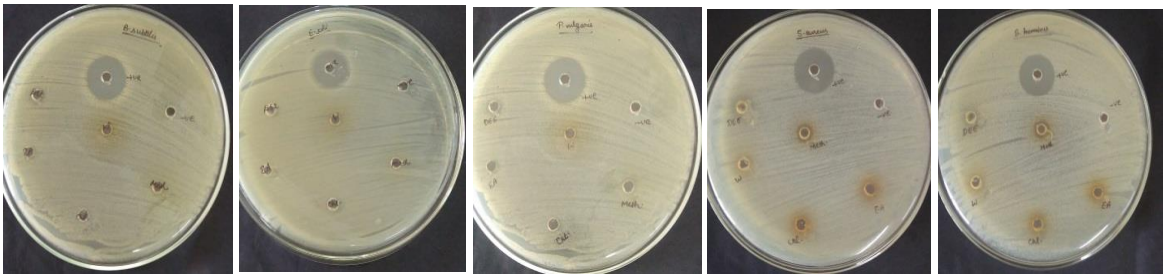


Figure 5: Antibacterial activity of different solvent (Methanol, Chloroform, Ethyl Acetate, Di-ethylether and Aqueous extracts of *in vitro* regenerated *T. cordifolia* plant against (a) *B. subtilis* (b) *E. coli* (c) *P. vulgaris* (d) *S. aureus* (e) *S. hominis*

Table 4 Antibacterial activity of wild plant, callus tissue and *in vitro* regenerated plant extracts

Bacterial Strains	Zone of Inhibition(cm)																	
	Wild plant						Callus tissue						*In vitro* regenerated plant					
	+ve Control	Met	EA	Chl	W	DEE	+ve Control	Met	EA	Chl	W	DEE	+ve Control	Met	EA	Chl	W	DEE
B. subtilis	3.4	1.4	1.2	-	1.5	-	3.4	1.0	1.7	-	-	-	3.4	-	-	-	-	-
E. coli	3.4	1.5	1.2	1.4	1.2	-	3.4	-	-	-	-	-	3.4	-	-	-	-	-
P. vulgaris	3.4	1.6	-	1.5	1.3	1.6	3.4	-	-	-	-	-	3.4	-	-	-	-	-
S. aureus	3.4	1.6	1.6	1.5	1.2	1.4	3.4	1.6	-	-	2.1	-	3.4	1.6	-	-	-	-
S. hominis	3.4	1.4	1.3	-	0.6	-	3.4	2.0	1.5	1.6	2.1	1.9	3.4	1.5	1.0			

DPPH assay

After phytochemical screening of medicinal plants, it was observed that the plant extracts contained a wide variety of free radical scavenging molecules. Some previous study also specified that phenols, flavonoids and tannins have been responsible for the antioxidant activity of various medicinal plants [44-46]. Various methods are available for the detection of free radical scavenging potential of the sample extracts, but the DPPH assay received maximum consideration due to its ease of use and convenience. In our study, the free radical scavenging potential of all plant sample extracts were analyzed by DPPH assay.

In current study various solvents (methanol, chloroform, ethyl acetate, and water) were used for extracting antioxidant compounds from wild plant, callus tissue and *in vitro* propagated *T. cordifolia*. The results revealed that the free radical scavenging activity of plant extracts was less than the ascorbic acid which was taken as control. The TAA% value of wild plant, callus tissue and *in vitro* raised plant extracts in various solvents (methanol, ethyl acetate, chloroform, diethyl ether& water) extracts were (89.26%, 83.27%, 76.28%, 60.67%and 81.11%), (48.45%, 51.52%, 40.78%, 32.00% and 35.27%) and (33.78%, 24.67%, 12.52%, 11.22% and 20.24%)and of Ascorbic acid (control) was 94.96%.The methanol extract of wild and *in vitro* regenerated plant, ethyl acetate extract of callus tissue showed maximum inhibition (89.26%, 33.78% and 51.52%) than other solvent extracts as shown in figure 6. Among the different solvents used, methanol exhibited highest amount of extractable compounds than ethyl

acetate, chloroform and water solvent in wild and *in vitro* regenerated plant while in case of callus tissue, the ethyl acetate extract displayed maximum antioxidant activity compared to remaining solvent extracts. The results revealed that the callus tissue and *in vitro* regenerated plantlets also exhibited free radical scavenging activity but less compared to the wild plant extracts, which can be further standardized by tissue culture skills.

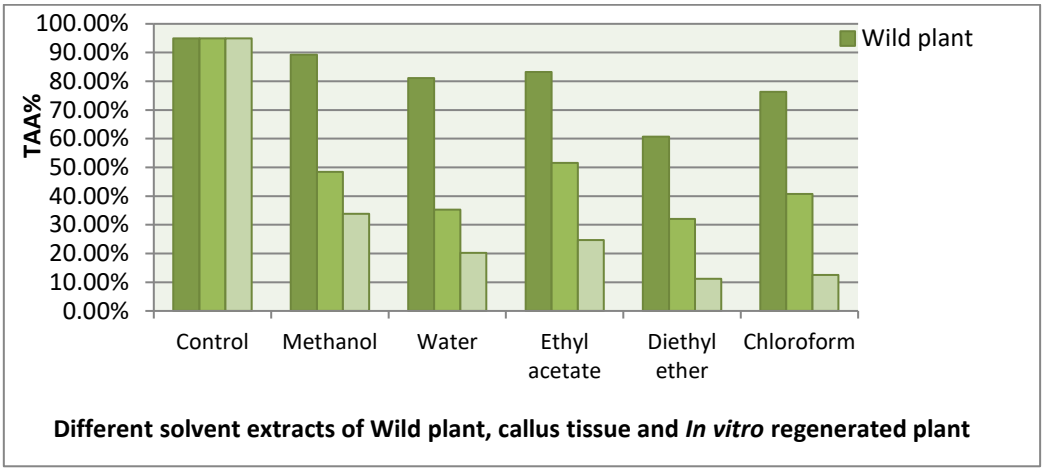


Figure 6 Antioxidant activity of different solvent extracts of wild, callus and *in vitro* regenerated plant extracts.

4. CONCLUSION:

Results of phytochemical analysis, antimicrobial and antioxidant activity estimation of wild and tissue culture raised *Tinospora cordifolia* plant displayed the presence of various phytoconstituents in parental explants (wild stem) as well as in their *in vitro* raised counterparts which proved that they retain their parental characteristics of phytochemical biosynthesis which can be enhanced further by altering the physical and hormonal parameters of cultural environment. So, the utilization of tissue culture technology for enhanced secondary metabolite production of *Tinospora cordifolia* appears promising and beneficial as many of the curative properties of this plant depend on these bioactive metabolites. These findings supported the use of *T. cordifolia* as multipurpose medicinal plant.

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