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Comparative assessment of antibacterial activity of *Tinospora cordifolia* (Willd.) Miers leaves and its callus

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ABSTRACT

Tinospora cordifolia (Willd.) Miers (family: Menispermaceae) is well-known for its versatile biological properties and has been extensively subjected by numerous researchers for its phyto-chemical evaluation to exploit its therapeutic potential to its maximum. Efforts have also been aimed to enhance the yield of secondary metabolites from the plant for obvious benefits. Plant tissue culture is one such technique which has been frequently employed to serve the purpose. In the present study, a comparative assessment has been carried out by subjecting leaves obtained from Tinospora cordifolia and its callus to the preliminary phytochemical as well as microbial evaluation. The MS medium supplemented with 2,4-D + 6-BA + IBA (0.5 ppm each) was found to be the best combination for the initiation and development of the callus on leaf explants. At 2 mg and 4 mg concentration of callus extract, inhibition was obtained for both the organisms. However, equal concentration of leaf extract showed inhibition of E. coli only.

Key words: *Tinospora cordifolia*, Callus, Antibacterial activity

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INTRODUCTION

*Tinospora cordifolia (*Willd.) Miers is a large, glabrous, deciduous climbing shrub belonging to the family *Menispermaceae* which is distributed throughout tropical Indian subcontinent including China, Sri Lanka, Nepal and Pakistan [1-3]. The notable medicinal properties reported are anti-diabetic, anti-periodic, anti-spasmodic, anti-microbial, anti-inflammatory, anti-arthritic, antioxidant, skin diseases, anti-allergic, anti-stress, anti-leprotic, antimalarial, hepatoprotective, immunomodulatory, anaemia, urinary disorder and antineoplastic activities [4-6]. The pharmacological activity of *Tinospora cordifolia* (*T. cordifolia*) is related to several classes of secondary metabolites, for example alkaloids [7], glycosides [8] diterpenoid lactones [9], steroids [10], sesquiterpinoids [11] and aliphatic compounds [12]. In Hindi, the plant is commonly known as Giloya, which is a Hindu mythological term that refers to the heavenly elixir that have saved celestial beings from old age and kept them eternally young. The stems of *T. cordifolia* are rather succulent with long filiform fleshy aerial roots from the branches. The bark is creamy white to grey, deeply left spirally, the space in between being spotted with large rosette like lenticels. The leaves are membranous and cordate. The flowers are small and yellow or greenish yellow. In auxiliary and terminal racemes or racemose panicles, the male flowers are clustered and female are usually solitary. The drupes are ovoid, glossy, succulent, red and peasized. The seeds are curved. Fruits are fleshy and single seeded. Flowers grow during the summer and fruits during the winter [13, 14].

The root, stem, leaves and starch of the plant are used for medicinal purpose, externally. The medicated oil of the plant is effectively used to reduce the pain and edema, in gout and skin diseases. It is a natural blood purifier and is very useful for skin problems like acne, psoriasis and eczema. It is immensely helpful in the digestive ailments like

hyperacidity, colitis, worm infestations, and loss of appetite, abdominal pain, excessive thirst, vomiting and liver disorders like hepatitis. Leaves of this plant contain 11.2% protein and are the rich source of calcium and phosphorus which producing the anti-oxidant activity *in vitro* models [15-17]. The phytoconstituents in the extracts were identified by treating the extract with various chemical reagents [18].

Various reports on its multiple medicinal use attracted attention for commercial exploitation of the plant to meet the requirements of the growing pharmaceutical industry. *T. cordifolia* natural stands are now fast disappearing and are threatened due to indiscriminate collection and over-exploitation. Conventional vegetative propagation of this plant has limited potential for large scale cultivation. Micropropagation technique can be most useful for its mass propagation as well as for its conservation. This paper highlights the results of an *in vitro* study of this plant.

In this paper, we describe the procedure to establish large scale production of biomass containing useful secondary metabolite by defining nutritional requirement and ensuring proper environmental conditions for their growth. The main objective of the study was to initiate a static culture using young leaves of *T. cordifolia* as an explant and anti-bacterial activity of 70 % methanolic extract of mature leaves of *T. cordifolia* as well as its callus culture.

MATERIALS AND METHODS

The explants were collected *in vivo* grown plant from Herbal Garden, Jamia Hamdard, Hamdard Nagar, New Delhi, India. The experimental materials such as leaves were obtained from 10-12 months old plants. For raising cultures, the explants leaves were washed for several times under running water with liquid detergent, Polyoxyethylene Sorbitan Monolaurate (5 % teepol), by thorough washing with distilled water (4-5 times). The leaves of *T. cordifolia* were surface sterilized with 0.1 % (w/v) mercuric chloride for 5 minutes followed by repeated washings with sterile double distilled water. After mixing all stock solutions (for preparation of MS media), 3% sugar was added and the pH of the media was adjusted to 5.8 with 0.1 N NaOH or HCL using digital pH meter before sterilization.

The Difco-bacto agar (0.8%) was dissolved and the medium was dispensed in the test tubes and capped with aluminium foil. Media were then autoclaved at 121ºC at 15 psi for 20 minutes. After surface sterilization, the explants were cut into small pieces (1 cm^2 for leaves), inoculated into the MS media supplemented with various combinations and concentrations of growth hormones. All inoculations and aseptic manipulations were carried out in a laminar airflow cabinet. All cultures were grown in an air-conditioned culture room illuminated by 40 watt white fluorescent tubes with 1000 lux intensity and 50-60% relative humidity. The photoperiod was maintained as 16 h light and 8 h dark. The temperature of the culture room was maintained at 25±2ºC. Visual observation of culture was made every 2-3 days. The initiated calli were separated aseptically from the mother explants and were transferred to the same medium on which they were initiated in order to develop the independent calli. During the maintenance, the sub-culturing was routinely done after every three weeks in order to maintain their growing ability. The 150 days old callus was used for further phytochemical investigation.

Screening for antibacterial activities

For the study of Antibacterial activity, the fresh leaves were collected and were washed under running tap water. The leaves and calli induced from leaf explants were dried at room temperature for one week to make it coarse powdered and stored in an air tight container. Powdered samples were refluxed with 70% methanol. The extractions were carried out for 2 hours at 50ºC temperature with mild shaking. The collected extracts were filtered through filter paper (Whatman No.41) and concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd, U.K) to get a viscous mass at 45ºC. The viscous masses were then kept at room temperature under the ceiling fan to get dried extract. Both extracts were redissolved in DMSO (Dimethyl Sulphoxide) to give final concentration of 100 mg/ml each. The extracts were kept under sterile conditions at 4°C.

Antibacterial activities were carried out by agar disc diffusion method. Three different bacterial cultures were used for testing Antibacterial activity. Different cultures namely *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis* were obtained from Microbiology Laboratory, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India. Activity of the above mentioned extracts was tested separately using Agar-well diffusion method [19, 20].

The medium was sterilized using an autoclave at 121ºC (15 lb/in^2). About 30 ml of the medium (Nutrient Agar Medium) with respective strains of bacteria was transferred aseptically in to each sterilized petri plate. The plates were left at room temperature for solidification. Each plate, a single well of 6 mm diameter was made using a sterile borer. The samples and control were placed in 6 mm diameter well. Plates were incubated at 37±2ºC for 24 hrs. Standard disc (6 mm diameter) with Streptomycin was used as positive control for anti-bacterial activity. Each

experiment was carried out in triplicate and diameter of the zone of inhibition was measured. Observations and results are shown in table 1.

RESULTS AND DISCUSSION

For the callus development, the leaves were surface sterilized with 0.1 % Mercuric chloride with a contact period of 5 minutes. The MS medium supplemented with 2,4-D + 6-BA + IBA (0.5 ppm each) was found to be the best combination for the initiation and development of the callus on leaf explants. Qualitative chemical tests showed that the natural leaf and cultured tissue contain alkaloids, glycosides, carbohydrates, proteins, phenolic compounds, flavonoids, steroids, saponins and tannins. Thus further work can be carried on the isolation procedure for finding out the exact moiety responsible for the biological activity.

The induction of friable, whitish mass of callus occurred from leaf explants on MS basal medium (Figure 1). Callus proliferated readily and retained its globular appearance even after sub culturing on semisolid growth regulator free MS medium up to ten folds within 4 weeks showing mass of callus (Figures 1). The 70% methanolic extracts of both leaves and callus induced from such leaves of *T. cordifolia* were assayed against three different bacterial cultures. The inhibition zones were obtained for *E. coli* and *S. aureus* (Table 1). At 2 mg and 4 mg concentration of callus extract, inhibition was obtained for both the organisms. However, equal concentration of leaf extract showed inhibition of *E. coli* only.

Table 1: Antibacterial activity of leaf and callus induced from leaf explant of *Tinospora cordifolia*

Micro-organism	Zone of inhibition in mm					
	Leaf extract (mg)		Callus extract (mg)		Streptomycin (%)	
	2	4	2	4	1	2
Staphylococcus aureus	-	-	14	22	8	18
Bacillus subtulis	-	-	-	-	6	12
Escherichia coli	15	22	13	24	17	26

All values of the mean of three replicate. - = no inhibition

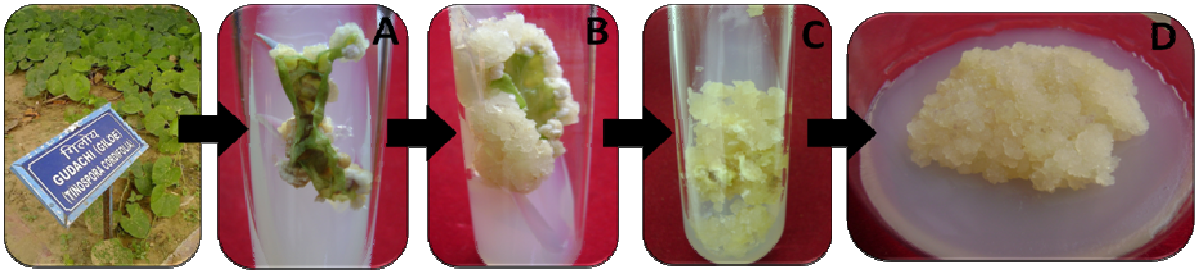

Figure.1: A to D: Stepwise establishment of callus of *Tinospora cordifolia*

CONCLUSION

Encouraging results obtained during the comparative phytochemical and biological evaluation of callus extract and natural leaf extract of *T. cordifolia* strongly supports the view that plant tissue culture technique continues to offer a valuable tool in determining the antibacterial activity. Despite the presence of similar phytochemical constituents in both callus extracts and natural leaf extract, the callus extract exhibited supra additive antibacterial activity against the one showed the leaf extract.

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