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Review Article

In Vitro Propagation and Medicinal Attributes of *Tinospora Cordifolia*: A Review

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Abstract

Concern on various aspects of medicinal plants is gaining momentum and research focus is increasing on a wide spectrum of activities starting form *in vitro* propagation to metabolomics. *Tinospora cordifolia* (Willd.) Miers ex Hook F & Thoms, is one of the important medicinal plant species widely used for various medicinal purposes particularly in India. However, due to large scale exploitation for commercial purpose, *Tinospora cordifolia* is facing rarity in natural habitats of India. This review includes the aspects of *in vitro* propagation and medicinal attributes including anti-microbial properties of *Tinospora cordifolia.* Biomedical study on the species and information based on metabolomics besides other biotechnological investigations including molecular characterization of diverse germplasm constitute the future scope of the review.

Keywords: *Tinospora cordifolia*; Micropropagation; Medicinal attributes; Guduchi; Antimicrobial

Introduction

Medicinal plants are considered as green gold owing to their invaluable contribution to the health care and wellbeing of human societies. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [1]. The use of medicinal plants with traditional know how is a much prevalent medicinal practice in India. Medicinal plants are widely used by all sections of the Indian population and it has been estimated that, in total over 7500 species of plant species are used by a number of ethnic communities [2,3]. In recent years, concern on various aspects of medicinal plants is gaining momentum and research focus is increasing on a wide spectrum of activities starting form *in vitro* propagation to metabolomics.

Tinospora cordifolia (Willd.) Miers ex Hook F & Thoms, is one of the important medicinal plant species widely used for various medicinal purposes particularly in India (Figure 1,2). The species is commonly known in India as 'Guduchi' (Sanskrit), 'Giloy' (Hindi) and 'Saguni lota' (Assam). It is a large deciduous climbing shrub belonging to the family *Menispermaceae*. It is distributed throughout the tropical Indian sub-continent ascending to an altitude of 300m. Due to its medicinal importance, *T. cordifolia* has been highly exploited for commercial purposes. At present the species is facing rarity in natural habitats of India. Recently, the National Medicinal Plant Board (NMPB) of India has prioritized this species for mass multiplication. In this context, biotechnological methods are considered as inevitable to promote commercial level cultivation of this species. This review encompasses the tissue culture propagation, chemistry and antimicrobial properties of *Tinospora cordifolia*.

Medicinal attributes of *Tinospora cordifolia*

Tinospora cordifolia is used for a number of medicinal purposes (Table 1). The plant is extensively used in Ayurvedic system of medicine for its general tonic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-allergic and anti-diabetic properties. The plant is also used in Ayurvedic 'Rasayanas' to improve the immune system and the body resistance against infections. The root of this plant is known for its anti-stress, anti-leprotic and anti-malarial activities [4]. The plant is mentioned in Ayurvedic literature as a constituent of several compound preparations, used in general debility, dyspepsia, fevers and urinary diseases. Antiviral properties against Ranikhet disease in poultry have also been ascribed to this climber [5].

The stem of *Tinospora* is bitter, stomachic, diuretic [6]; stimulates bile secretion; causes constipation; allays thirst, burning sensation and vomiting; enriches the blood and cures jaundice. The extract of the mature stem is useful in skin diseases [7,8]. The root and stem of *T. cordifolia* are prescribed in combination with other drugs as an anti-dote to snakebite and scorpion sting [5,9]. Dry barks have anti-spasmodic, anti-pyretic [10], anti-allergic [11], anti-inflammatory [12,13] and anti-leprotic properties [14]. Decoction of the leaves was reported to be used for treatment of gout and young leaves were used as a liniment in erysipelas [15]. Root is a powerful emetic and used for visceral obstruction, its watery extract is used in leprosy. Pulverized fruit is used as a tonic and also for jaundice and rheumatism [16]. This

Figure 1: Fruiting twig of *Tinospora cordifolia.*

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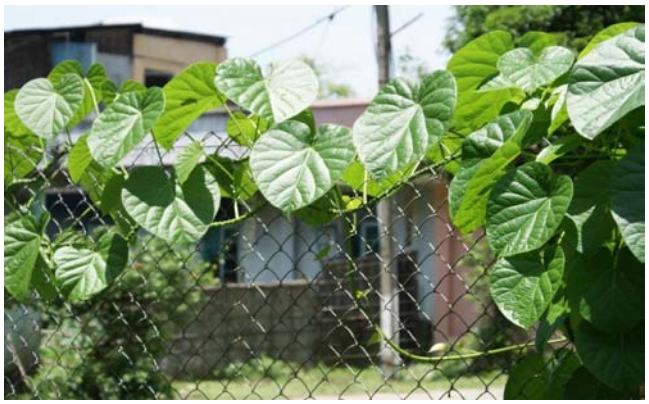

Figure 2: Tender twig of *Tinospora cordifolia* creeping on iron fencing.

plant species is reported as for treating diabetes [17-19]. *Tinospora cordifolia* has been reported to treat throat cancer in humans [20]. The plant species has also been reported to show efficacy in the treatment of allergic rhinitis [21].

In a recent review, the genetic diversity of the plant and active components isolated from the plant and their biological role in disease targeting was discussed thoroughly [22]. Another comprehensive review describes the medicinal prominence of *Tinospora cordifolia* in therapeutics as well as its morphology, growth constraints, biochemical composition, biological activities, and the future prospects of this important medicinal plant species [23]. An inclusive review is also available on various properties and medicinal uses of *T. cordifolia* described in Ayurveda, along with phytochemical and pharmacological reports [24].

In vitro propagation of *Tinospora cordifolia*

Development of plant tissue culture technology offers a great potential for rapid multiplication of plant germplasm. It serves as a powerful tool for short to medium term conservation of important plant species. Tissue culture technology provides an ideal way for large-scale propagation and the reintroduction of the plants in its natural habitats [25,26]. *In vitro* propagation increases the efficiency and scales up plant production [27]. Moreover, plant cell and tissue culture, as well as genetic engineering may be an alternative to the conventional method for the improvement of medicinal plants [28]. The *in vitro* cultures could be preserved overtime and multiplied as and when required [29]. Tissue culture also facilitates the exchange of germplasm within and across the countries [30].

There are several literatures available on *in vitro* propagation of *Tinospora cordifolia* (Table 2). In most of the work, nodal explants have been used in MS basal media [31] with various combination and concentration of plant growth hormones. In a recent experiment, development of multiple shoots in high frequency was achieved in nodal explant culture of *Tinospora* cordifolia [32]. MS basal medium was used throughout this investigation with different combinations and concentrations of Benzyl Amino Purine (BAP), Kinetin and Thidiazuron (TDZ).Combination of BAP (2mg/l), Kinetin (4mg/l) and TDZ (0.20mg/l) in MS medium gave a maximum average of 10.29 numbers of shoots per explant within 30 days of inoculation. Root initiation was noticed after 5-6 days in half strength MS medium supplemented with IBA (2mg/l) and it became profuse after 5 weeks. The rooted plantlets were transplanted *ex vitro* and rose in pots under green house conditions for one month followed by their field transfer. Earlier, a micropropagation protocol was developed using *Tinospora cordifolia* collected from North East India [33]. MS basal medium was used throughout this experiment with different combination and concentrations of BAP, Kinetin and IAA.

A protocol of micropropagation of *Tinospora cordifolia* was reported where various explants (shoot tip, axillary bud and cotyledonary node) were cultured on MS medium supplemented with different concentrations of plant growth hormone [34]. In this work, kinetin at 3.0 mg/l proved to be the best for shoot induction. Axillary bud and cotyledonary node explants showed good response when compared with shoot tip explants. Shoot elongation was found to be best in MS medium containing Kinetin (3.0mg/l) and Gibberellic Acid (0.5mg/l). Method was also optimized for controlling of phenol exudation in the culture using Polyvinylpyrrolidone (PVP).

In vitro multiplication of *T. cordifolia* via direct somatic

Table 1: Medicinal attributes of *Tinospora cordifolia.*

Plant parts	System of medicine/ test method	Biological Activity	References
Whole plant	Ayurvedic (India)	Improve the immune system and the body resistance against infections.	[4]
Roots	Traditional	Anti-stress, anti-leprotic and anti-malarial	[4]
Whole plant	Bioassay	Antiviral properties against Ranikhet disease in poultry	[5]
Stem	Bioassay	Anti-stress, anti-leprotic and anti-malarial	[6]
Stem	Ayurvedic (India)	Stimulates bile secretion; causes constipation; allays thirst, burning sensation and vomiting; enriches the blood and cures jaundice; useful in skin diseases	[7,8]
Root and stem	Traditional	Anti-dote to snakebite and scorpion sting	[5,9]
Dried bark (stem)	Bioassay	Anti-spasmodic, anti-pyretic, anti-allergic, anti-inflammatory and anti-leprotic.	[10,11,12, 13,14]
Leaf	Traditional	Treatment of gout and as a liniment in erysipelas	[15]
Fruit	Traditional	As tonic and also for jaundice and rheumatism	[16]
Whole plant	Bioassay	Antidiabetic	[17,18,19]
Whole plant	Bioassay	Treatment of throat cancer in humans	[20]
Whole plant	Bioassay	Treatment of allergic rhinitis	[21]
Whole plant	Bioassay	Antimicrobial	[46, 47, 48, 49, 50, 51, 52, 53]

Table 2: *In vitro* propagation of *Tinospora cordifolia.*

Activity	Explant	Media	Growth regulator	References
Micropropagation	Nodal segment	MS	Thidiazuron, BAP, Kinetin, IAA	[32, 33]
Micropropagation	Axillary bud and cotyledonary node	MS	Kinetin, GA_3	[34]
Somatic embryogenesis	Leaf	MS	2.4-D	[35]
Clonal propagation	Nodal segment	MS, WPM	Kinetin, BA	[36]
In vitro regeneration	Nodal segment	MS	Triacontanol, kinetin	[37]
Callus formation	nodal segments, leaf and inter-node	MS	Kinetin	[38]
Callus formation	Leaf	MS	2.4-D, Kinetin	[39]
Multiple shoot	Nodal segment	MS	BA, NAA	[40, 41]
In vitro berberine production	Cell suspension	Linsmaier and Skoog's	-	[42]
In vitro berberine production	Leaf, petiole and stem derived calli	MS	NAA, Kinetin, BA	[43]
In vitro berberine production	Hairy root culture	MS	-	[44]

embryogenesis using leaf explants of 15 days old plants on MS medium supplemented with 2,4-D (0.5mg/l) and glutamine (20mg/l) produced viable somatic embryos [35]. Another protocol was developed for rapid clonal propagation of *Tinospora* through *in vitro* culture of mature nodal explants [36]. Shoots were initiated on both MS medium and Woody Plant Medium (WPM) supplemented with 2.32 µm Kinetin. Of the two basal media tested, WPM was found to be superior to MS medium for the induction of multiple shoots. Among the cytokinins tested, Benzyl Adenine (BA) was more effective than Kinetin for axillary shoot proliferation. Nodal explants were reported as best explants for *in vitro* regeneration of *Tinospora* [37]. In this study, a combination of 11.38µM Triacontanol [CH3(CH2)28CH2OH] and 13.94µM Kinetin was reported as best shoot inducing hormone combination. However, the influence of different auxins and their combination with cytokinin was not effective for shoot proliferation from nodal explants.

In *Tinospora* tissue culture, callus formation was observed from nodal segments, leaf and inter-node explants when planted on different combinations of hormones in MS Medium. However only nodal explants showed better shoot growth in MS medium containing kinetin (1.5 mg/l). Roots were developed in the medium containing 1.0mg/l BAP (1.0mg) and 2.5mg/l Naphthaleneacetic Acid (NAA) [38].

Induction of callus was also obtained from leaf explants while culture in MS medium with 2,4-D alone or in combination with kinetin. However such callus failed to differentiate. Direct shoot induction was achieved from nodal explants culture in MS medium supplemented with kinetin (8µM) or in combination of kinetin and BAP (12 and 2µM respectively). The microshoots developed roots in medium fortified with NAA (8µM) [39].

Regeneration of multiple shoot was also obtained from nodal segments of *Tinospora* in MS basal medium supplemented with the combination of BA (0.5 mg/l) and NAA (0.2mg/l). Regenerated shoots were rooted on half strength MS basal medium containing both BA (1.0mg/l) and IAA (0.2mg/l). Rooted plantlets were transferred to pots containing soil for acclimatization, for a period of three weeks and were successfully established in soil [40]. The shoot proliferation was also observed in MS medium containing BA and kinetin. While rooting of the microshoots was obtained in half strength MS medium supplemented with 0.4mg/l NAA [41].

Production of active principle through *in vitro* culture also draws attention of the scientific communities. Berberine, an isoquinolene alkaloid, together with its related analogs protoberberine and palmatine were detected in cell suspension cultures derived from leaf explants of *Tinospora cordifolia*. Berberine production was achieved in an optimized Linsmaier and Skoog's medium with specific pH, plant growth regulators and carbon sources. The yield of berberine in cell suspensions of *Tinospora* was reported as 5-14-folds higher than that of intact plant [42].

In an attempt to up gradation of the content of berberine in *Tinospora*, through biotechnological interventions, four week old leaf, petiole and stem derived calli of *Tinospora* was sub-cultured on to MS medium, supplemented with various growth regulators. MS medium with NAA (2 mg l^{-1}) supplemented with BA or kinetin, each at 2 mg/l, was identified as the basal production medium for *in vitro* production of berberine, yielding 7.55 µg and 7.36 µg berberine respectively, per gram of calli. Calli produced from stem segments registered maximum amount of berberine compared to leaf and petiole derived callus cultures [43].

Protoberberine alkaloids were successfully isolated from the hairy root cultures of *Tinospora cordifolia* transformed with *Agrobacterium rhizogens*. Hairy roots of *Tinospora cordifolia* were induced from the shoot cultures by transformation with *Agrobacterium rhizogenes* on a solid YMB medium. Roots were sub-cultured on liquid MS medium containing B5 vitamins and 3% sucrose without hormone under an optimized growth condition. This study revealed a higher amount of berberine (0.034%) production in the cultures treated with 500mg/L of L-Tyrosine as precursor, than the control [44].

It is to be mentioned that extensive chemical investigations have been made on *Tinospora cordifolia* and quite a good numbers of constituents have been isolated so far. The isolated constituents mainly belong to diverse classes such as alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides [45].

Antimicrobial activities of *Tinospora cordifolia*

Tinospora cordifolia has also been reported to have efficacy in the treatment of various microbial diseases (Table 1). More particularly, the plant shows considerable antimicrobial activities against several disease-causing microorganisms. A study on the antibacterial

activity of the aqueous, ethanol and chloroform extracts of *Tinospora cordifolia* by disc diffusion method against a number of gram positive and gram-negative bacteria revealed its significant antibacterial activity. This finding justifies the uses of *Tinospora cordifolia* in traditional medicine to treat various infectious diseases [46].

The methanol extracts of *Tinospora cordifolia* have been reported to have potential against microbial infections. Plant extracts showed activity against a number of bacterial species viz., *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Shigella flexneri, Salmonella paratyphi, Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacter aerogene*, and *Serratia marcesenses* [47,48]. In mice models, Tinospora extract has been reported to function in bacterial clearance and improved phagocytic and intracellular bactericidal capacities of neutrophils [49].

Tinospora stem extract obtained through soxhlet extraction and separated by various chromatographic techniques using mixed solvent system, showed antibacterial effect against *Escherichia coli, Staphylococcus aureus* and *Staphylococcus albus* [50]. Hydro-alcoholic extract of *Tinospora cordifolia* creeped on *Azadirachta indica* tree showed potential antimicrobial activity similar to *Azadirachta indica.* The antibacterial activity was recorded against *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Pseudomonas sp, Aspergillus niger, Aspergillus fumigates, mucor sp* and *Pencillium* [51]. Antibacterial activity of *Tinospora cordifolia* has also been reported on urinary tract pathogen [52]. In a recent work, antibacterial activity of silver nanoparticles synthesized from stem of *Tinospora cordifolia* were analysed against multidrug-resistant strains of *Pseudomonas aeruginosa* isolated from burn patients. Silver nanoparticles from *Tinospora cordifolia* possess very good antibacterial activity which makes them a potent source of antibacterial agent [53].

Conclusion

Tinospora cordifolia is emerging as a multipurpose plant with diverse medicinal attributes. Due to its enormous medicinal and phytochemical importance, this plant species has been highly exploited for commercial purposes. At present the species is facing rarity in natural habitats of India. The species deserve more evaluation for its therapeutic value using sophisticated tools and techniques. This review highlighted only a few aspects of investigation on this plant species. Biomedical study on the species and information based on metabolomics besides other biotechnological investigations including molecular characterization of diverse germplasm constitute the future scope of the review.

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