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

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Received: September 12, 2014; Accepted: October 15, 2014; Published: October 17, 2014

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 from 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

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-arthritis, 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

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.
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 greenhouse 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

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**Table 1: Medicinal attributes of Tinospora cordifolia.**

<table>
<thead>
<tr>
<th>Plant parts</th>
<th>System of medicine/ test method</th>
<th>Biological Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole plant</td>
<td>Ayurvedic (India)</td>
<td>Improve the immune system and the body resistance against infections.</td>
<td>[4]</td>
</tr>
<tr>
<td>Roots</td>
<td>Traditional</td>
<td>Anti-stress, anti-leprotic and anti-malarial</td>
<td>[4]</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Bioassay</td>
<td>Antiviral properties against Ranikhet disease in poultry</td>
<td>[5]</td>
</tr>
<tr>
<td>Stem</td>
<td>Bioassay</td>
<td>Anti-stress, anti-leprotic and anti-malarial</td>
<td>[6]</td>
</tr>
<tr>
<td>Stem</td>
<td>Ayurvedic (India)</td>
<td>Stimulates bile secretion; causes constipation; alleviates thirst, burning sensation and vomiting; enriches the blood and cures jaundice; useful in skin diseases</td>
<td>[7,8]</td>
</tr>
<tr>
<td>Root and stem</td>
<td>Traditional</td>
<td>Anti-dote to snakebite and scorpion sting</td>
<td>[5,6]</td>
</tr>
<tr>
<td>Dried bark (stem)</td>
<td>Bioassay</td>
<td>Anti-spasmodic, anti-pyretic, anti-allergic, anti-inflammatory and anti-leproptic.</td>
<td>[10,11,12, 13,14]</td>
</tr>
<tr>
<td>Leaf</td>
<td>Traditional</td>
<td>Treatment of gout and as a liniment in erysipelas</td>
<td>[15]</td>
</tr>
<tr>
<td>Fruit</td>
<td>Traditional</td>
<td>As tonic and also for jaundice and rheumatism</td>
<td>[16]</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Bioassay</td>
<td>Antidiabetic</td>
<td>[17,18,19]</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Bioassay</td>
<td>Treatment of throat cancer in humans</td>
<td>[20]</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Bioassay</td>
<td>Treatment of allergic rhinitis</td>
<td>[21]</td>
</tr>
<tr>
<td>Whole plant</td>
<td>Bioassay</td>
<td>Antimicrobial</td>
<td>[46, 47, 48, 49, 50, 51, 52, 53]</td>
</tr>
</tbody>
</table>
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 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 explants for shoot proliferation from nodal explants. Of different auxins and their combination with cytokinin was not an optimum combination. Tinospora [37]. In this study, a combination of 11.38µM Triacontanol [CH3(CH2)28CH2OH] and 13.94µM Kinetin was reported as best explants 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 isoquinoline 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. Calii 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 rhizogenes. 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, sesquiterpenoids, 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

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

<table>
<thead>
<tr>
<th>Activity</th>
<th>Explant</th>
<th>Media</th>
<th>Growth regulator</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micropropagation</td>
<td>Nodal segment</td>
<td>MS</td>
<td>Thidiazuron, BAP, Kinetin, IAA</td>
<td>[32, 33]</td>
</tr>
<tr>
<td>Micropropagation</td>
<td>Axillary bud and cotyledonary node</td>
<td>MS</td>
<td>Kinetin, GA₅</td>
<td>[34]</td>
</tr>
<tr>
<td>Somatic embryogenesis</td>
<td>Leaf</td>
<td>MS</td>
<td>2.4-D</td>
<td>[35]</td>
</tr>
<tr>
<td>Clonal propagation</td>
<td>Nodal segment</td>
<td>MS, WPM</td>
<td>Kinetin, BA</td>
<td>[36]</td>
</tr>
<tr>
<td><em>in vitro</em> regeneration</td>
<td>Nodal segment</td>
<td>MS</td>
<td>Triacontanol, kinetin</td>
<td>[37]</td>
</tr>
<tr>
<td>Callus formation</td>
<td>nodal segments, leaf and inter-node</td>
<td>MS</td>
<td>Kinetin</td>
<td>[38]</td>
</tr>
<tr>
<td>Callus formation</td>
<td>Leaf</td>
<td>MS</td>
<td>2.4-D, Kinetin</td>
<td>[39]</td>
</tr>
<tr>
<td>Multiple shoot</td>
<td>Nodal segment</td>
<td>MS</td>
<td>BA, NAA</td>
<td>[40, 41]</td>
</tr>
<tr>
<td><em>in vitro</em> berberine production</td>
<td>Cell suspension</td>
<td>Linsmaier and Skoog’s</td>
<td>-</td>
<td>[42]</td>
</tr>
<tr>
<td><em>in vitro</em> berberine production</td>
<td>Leaf, petiole and stem derived calli</td>
<td>MS</td>
<td>NAA, Kinetin, BA</td>
<td>[43]</td>
</tr>
<tr>
<td><em>in vitro</em> berberine production</td>
<td>Hairy root culture</td>
<td>MS</td>
<td>-</td>
<td>[44]</td>
</tr>
</tbody>
</table>
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 aerogenes*, and *Serratia marcesens* [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* creeping 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 *Penicillium* [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 in functional clearance and improved phagocytic and intracellular bactericidal capacities of neutrophils [49].

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