

Invitro propagation and Medicinal Properties of *Tinospora cordifolia*: A Review

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ABSTRACT

Traditional medicine has remained as the most affordable and easily accessible source of treatment in the primary healthcare system of resource poor communities in India. A huge interest always exists in exploring nutraceuticals from plant materials to replace synthetic drugs in order to overcome their adverse effects and also for economic reasons. *Tinospora cordifolia* (TC) is a large extensively spreading glabrous, perennial deciduous twiner with succulent stems and papery bark; leaves simple, alternate, cordate, entire, 7-9 nerved; flowers in clusters, female flowers usually solitary; fruits drupes, red when ripe. *Tinospora cordifolia* is an important drug of Indian systems of medicine and used in medicines since times immemorial. The drug is well known Indian bitter and prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has been also indicated useful in the treatment of heart disease, leprosy and helmenthiasis. The starch obtained from the stem is highly nutritive and digestive and used in many diseases. The present review aims to summarize the information concerning the invitro propagation, isolation and characterization of bioactive compounds and medicinal aspects of the *Tinospora cordifolia* plant.

1. Introduction

The past decade has witnessed a tremendous resurgence in the interest and use of plants as medicine throughout the world. Phytomedicine has gaining popularity as an alternative medicine. In ancient India, about 75% of the population was dependent on the traditional system of medicine known as Ayurveda. Currently the ayurvedic practitioners have identified a number of medicinal preparations and surgical procedures for curing various ailments and diseases.

Miers Hook F. and Thoms reported *Tinospora cordifolia* (wild), is widely used in folklore and Ayurvedic system of medicine, belonging to the family Menispermaceae (Kashyap *et al.*, 2015). It is commonly known as Guduchi. It is a glabrous climbing shrub with heart shaped leaves and distributed throughout the tropical Indian subcontinent, Bangladesh, Sri Lanka, South Africa (The wealth of India, 1959). In Ayurveda, it is given to the patients suffering from fever, diabetes, jaundice, skin disease, urinary diseases and piles (Sharma and Vashistha, 2014). The pharmaceutical significance of this plants is mainly because of various bioactive compounds such as alkaloids, carbohydrates, Glycosides, proteins and aminoacids, fixed oil and fats tanins, saponin, steroids (phytosterols), flavonoids, phenols etc (Sivakumar and Dhana ., 2011) found in this plant. Due to its medicinal importance, it has been highly exploited for commercial purpose. The present review aims to summarize the information concerning the invitro propagation, isolation and characterization of bioactive compounds and medicinal aspects of the *Tinospora cordifolia* plant.

2. Classification of *Tinospora cordifolia*:

Kingdom:	Plantae/
Division:	Magnoliophyta
Class:	Magnoliopsida

Order:	Ranunculale.
Family:	Menispermaceae
Genus	<i>Tinospora</i>
Species	<i>cordifolia</i>

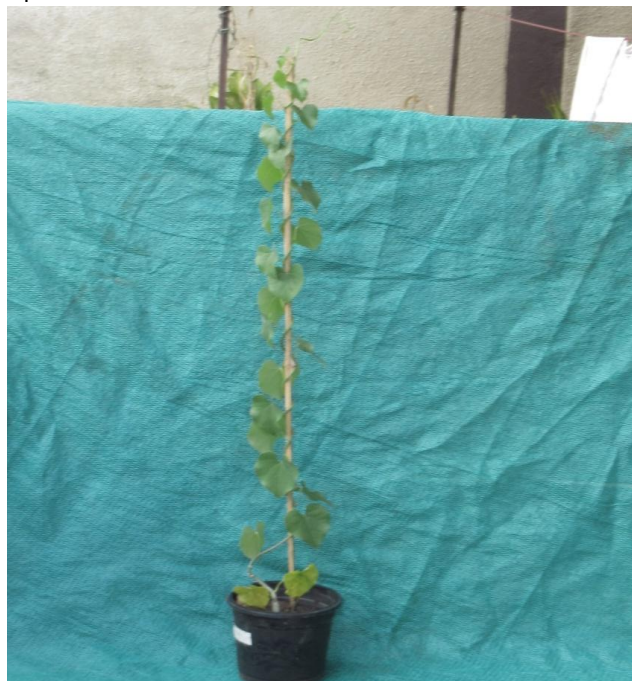


Fig. *Tinospora cordifolia* plant grown in pot

3. Morphological characteristics:

Tinospora cordifolia is a deciduous climbing shrub with several twining branches (Shrivastava, *et al.*, 2013). The bark is papery, creamy white to grey in appearance with large rosette-like lenticles. Leaves are simple, alternate lobbed cordate, entire, 7-9 nerved; Flowers are small, cymose, yellow or greenish in colour. Fruits are pea shaped, shiny, druping and become red when fully grown. Flowers occur in summer;

and fruits grow during winter. The leaves afford a good fodder for cattle (Singla and Singla, 2010). It has tubercles on the surface of grayish stem. Leaves are broad and heart shaped. Small yellow flowers are present on long spikes (Sangeeta *et al.*, 2013). Seeds are usually hooked or reniform. Roots are aerial, thread-like, long, fleshy and arise from the branches (Spandana, *et al.*, 2013).

4. Growth Requirement:

Tinospora cordifolia is a climber so it requires support for its growth. Fast growing species such as Neem (*Azadirachta indica*), jatropha (*Jatropha curcas*) and Moringa (*Moringa oleifera*) have been planted to provide support for its growth (Shefali and Nilofar., 2013). Giloy can be grown in all most all climates but prefers warm climate. Planting is usually done during rainy season (July to August). It prefers medium black or red soil for cultivation. Giloy can also be successfully grown in large variety of soils, ranging from sandy to clay loam. However, the soil should be well drained with organic matter for its growth (Mittal, *et al.*, 2014).

5. In vitro propagation of *Tinospora cordifolia*:

Large number of modern drugs has been isolated from natural sources many based on their use in traditional medicine (Ruikar *et al.*, 2009). Therefore the in vitro or micropropagation protocols for elite stock of *Tinospora cordifolia* are urgently needed. Micropropagation ensures not only continuous supply of plant throughout the year but also prevents destruction of the natural population of medicinal plants. Bhat *et al.*, (2013) had regenerated multiple shoot from nodal segments of *Tinospora cordifolia* in MS basal media supplemented with the combination of BA (0.2mg/L) and NAA (0.5mg/L) proliferation of these shoots appeared from the node via axillary branching of buds from the explants. A little addition of NAA, 2mg/L into the MS medium with BA had reduced tremendously the formation of multiple shoots. The multiple shoots were isolated and transferred (after 4 weeks) to MS medium containing different concentrations of BA (0.20-1.0 mg/L) and IAA (0.2-1.5mg/L) for root induction. These rooted shoots were transferred to the plastic pots containing a mixture of soil and vermicompost. These plants were successfully established in soil

In vitro callus initiation and organogenesis from shoot tip explants of *Tinospora cordifolia* was observed (Sharma and Vaishtha. 2014). In this study, shoot tips were cultured on MS and WPM supplemented individually with IAA, NAA and 2,4-D at 0.5-4.0 mg/L concentration. Among the different growth regulators tried in *Tinospora cordifolia*, 2,4-D was most effective for callus induction. The callus so obtained was cultured on WPM supplemented with different concentrations ranging from (0.5-4.0mg/L) of BAP and KN for shoot formation. It was observed that 2 mg/L BAP Concentration was effective for callus organogenesis (Sharma and Vaishtha. 2014). Another experiment on in vitro propagation of *Tinospora cordifolia* was carried out in which vermicompost and its extracts along with coelomic fluid was used as tissue culture media, this study was aimed at using vermicompost, an organic manure, its extract from coelomic fluid (body fluid) of earthworm as an alternative to MS media to find out how best these preparations can contribute to micropropagation of *Tinospora cordifolia*

(Kashyap *et al.*, 2015). In this study, initiation of shoot cultures in *Tinospora cordifolia* appeared to be rather difficult initially due to heavy bacterial and fungal contamination. But treatment of explants with 0.01 HgCl₂ (W/V) for two minutes greatly helped to overcome this contamination. Callus induction rate was 98% in the vermin compost extract medium along with coelomic fluid. KIN along with auxins considerably enhanced callus growth. MS+BAP+NAA medium showed 100% callus induction, vermin compost only showed 75%, vermicompost extract only showed 100% and vermicompost extract (explants given coelomic fluid spray) containing medium showed 100% callus induction development (Kashyap *et al.*, 2015).

6. Isolation and characterization of bioactive compounds of *Tinospora cordifolia* :

In herbal drug discovery bioactive compounds of all medicinal plants have played an important role. *Tinospora cordifolia* is also one such medicinally important plant contained many chemical compounds and formed the basis of most important medicines. In this context, isolation and characterization of pharmacologically active compounds from *Tinospora cordifolia* continue till date.

Separation and quantitation of important markers was carried out earlier in which 20 beta -hydroxycyclopentanone, tinosporoside, cordioside and columbin were obtained from *Tinospora* species. These marker compounds isolated from 70% ethanolic extract of *Tinospora cordifolia* by repeated column chromatography are identified by mass spectroscopy (Ahamad *et al.*, 2006). In this study, the four marker compounds are separated on a Rp (RP-18, 5 µm, 250x 4.6mm i.d.) column using water- acetonitrile gradient. A typical HPLC-UV-DAD method was used for their detection. This study revealed that *Tinospora cordifolia* possess efficient levels of all the four bioactive compounds followed by *Tinospora crispa* and then *Tinospora malabrica* species.

Phytochemical parameters of powdered *Tinospora cordifolia* stem like total ash acid, insoluble ash, water soluble extractive value, alcohol soluble extractive value, was carried out. HPLC method was conducted for identification of phytoconstituents such as berberine, alkaloids, carbohydrates, Glycosides, protein and aminoacids, Fixed oils and fats, saponins, steroids, flavonoids and phenol. The results shows that the methanol extracts have higher concentration of berberine when compared to other solvent fractions like petroleum ether, chloroform etc. This study revealed that standardization profile and characterization of berberine compounds from *Tinospora cordifolia* would be immense value in botanical identification and authentication of plant drug (Sivakumar and Dhana., 2011). Phytochemical study of the stem extract of *Tinospora cordifolia* revealed the presence of alkaloids, carbohydrates, proteins, tanins, flavonoid, sterols, terpenes, glycosides and saponins. Sardhara and Gopal, (2013) carried out qualitative phytochemical screening was carried out by using standard procedures and results showed that alkaloids, terpenoid, flavonoid, phenols were exceptionally present in ethanolic extract of the plant but these phytochemicals were found to be absent in water extract The presence of these

metabolites signifies the potential of *Tinospora cordifolia* as a source of therapeutic agent.

Devinder et al.,(2014) carried out analysis of fresh stem of *Tinospora cordifo* for nutritional and phytochemical composition. The result of this study showed proximate mineral composition of *Tinospora cordifolia* as moisture 69%, crude fat 1.03%, carbohydrates 9.05%, Ash 3.6% and crude fibre 14.8% indicated that the *Tinospora cordifolia* is a rich source of fibre content (14.831%). Also vitamin C (17.01%) was also found in the stem of *T.cordifolia*. Estimation of berberine in stem of *Tinospora cordifolia* was done by HPLC (Srinivasan et al., 2008). The nutrient composition obtained from the analysis, carried out by Devindaret al.,(2014) suggested that this plant is a good source of antioxidants, vitamin C and macro and micro-nutrients make them strongly suitable to be incorporated into human nutrition.

The gas chromatography and mass-spectroscopy analysis of *Tinospora cordifolia* crude and purified stem revealed the presence of 40 compounds (Chloroform-15, methanol-14 and petroleum ether-11 (Albinjose et al., 2015). In this study, it was found that propanoic acid, 2-(3-acetoxy-4,4,14-trimethylandrosta-8-en-17-yl) was the common compound in crude and purified *Tinospora cordifolia* stem extract. Other compounds which were found in this investigation are Glycine, oleic acid phorbol, Dibutyl phthalate, Butenoic acid etc. Recently, a simple four step method for sequential extraction and quantification of *Tinospora cordifolia* leaf pigments and metabolites has been proposed (Pathan et al.,2015). Results related to this method of extraction estimates leaf pigments such as chlorophyll-a and chlorophyll-b and phyto-constituents like reducing sugars, soluble sugar, starch, proteins, Free aminoacids, phenols and flavonoids. Data with respect to chlorophyll pigments and carotenoids extracted with sequential extraction method and routine method (chlorophyll-a (mg g⁻¹) 1.41, chlorophyll b -0.36, chlorophyll a+b-1.76 and carotenoids 0.34 mg g⁻¹ indicated that *Tinospora cordifolia* leaf has substantial amounts of these pigments. It has been stated that regular intake of chlorophyll keeps digestive and circulatory system healthier. Also Banu, (2015) studied antioxidant activity of chlorophylls isolated from some plants and reported that *phyllanthusemblica* chlorophyll has better activity than ascorbic acid. Thus, extraction of chlorophylls, carotenoids, sugars, free aminoacids along with phenols and flavonoids in ethyl alcohol can be safer in utilization of these bio-molecules in human diet. Preliminary phytochemical screening of leaves and stem of *Tinospora cordifolia* revealed the presence of different bioactive secondary metabolites which might be responsible for their medicinal attributes (Sama and Sawaraj.,2012). Phytochemical screening of leaf and stem of *Tinospora cordifolia* indicated the presence of alkaloids, cardiac glycosides, tanins, phenols, carbohydrates and flavonoids and suggested that it is an important source of bioactive compound that may supply novel medicine (Jyoti Rani, et al., 2015).

7. Medicinal Aspects:

Anticancer activities:

Cancer is the second leading cause of death in the world, so scientists over the entire world do their best to discover safe

cancer therapy. Various bioactive compounds derived from medicinal plants have assisting for their efficacy and tolerability and treatment of cancer (Kumar and Jesudas. 2013). Among these medicinal plants *Tinospora cordifolia* also shows anti-cancer activity. This activity is mostly shown in animal models. Jagetia and Rao, (2006) studied that Dichloromethane extracts of *Tinospora cordifolia* shows cytotoxic effects owing to lipid peroxidation and release of LDH and decline in GST. Thippeswamy and Salimath, (2004) reported that hexane extract fraction of *Tinospora cordifolia* induced cell death against Ehrlich ascites tumor in mice. Data showed that the hexane extract fraction of *Tinospora cordifolia* is capable of inducing programmed cell death in Ehrlich ascites tumor in vivo. Upadhyay et al.,(2011) reported that root extract has widely affected radiation induced rise in lipid peroxidation and resulted in decline of GSH in tests in pre-irradiating mice

Tungpradit, et al.,(2010) carried out a study where the ethyl acetate extract of *Tinospora cordifolia* showed high antiproliferative activities against lung carcinoma cells, murine Lewis lung carcinoma (LLC) cells, A549 human lung carcinoma cells in the DNA fragmentation assay. Same concurrent study was carried out by Florida et al.,(2013). The result of DNA fragmentation assay reflecting the genotoxic potentials of *Tinospora cordifolia* leaf extracts against HT₂₉ colorectal carcinoma cell line. The DNA from HT₂₉ cells treated with ethyl acetate extract displayed genotoxicity. The highest damage of DNA was noticed in cells treated with 150 µg ethyl acetate extract. The result of above study provide rationale to develop *Tinospora cordifolia* leaves extract enriched with secondary metabolites into value added nutraceutical product for cancer prevention. Anticancer activity of extract of indigenous medicinal plants *Eucalyptus globulus* and *Tinospora cordifolia* grown in polluted and natural sources were investigated against human breast cancer (Kumari and Jesudas. 2013) cells Mc (F) and Tamoxifen was used as experimental control. A methodical evaluation of cytotoxicity effects revealed that individual methanolic extract of these two plants and their combined samples and tamoxifen showed dose dependent cytotoxicity against MC(f) -7 cell lines. Most anticancer drugs are designed to eliminate rapidly proliferating cancerous cells and therefore they show cytotoxicity and induce apoptosis in cancer cells (Kumar and Jesudas. 2013). Rachana and Kaur, (2013) reported the anti-brain cancer potential of methanolic extract of TC against C6 glioma cells. This study provides the first evidence for the presence of anti proliferative differentiation-inducing and anti-migratory, anti metastatic potential of *Tinospora cordifolia* in glioma cells and possible signalling pathways involved in its mode of action. Other studies have shown that the polysaccharide fraction of *Tinospora cordifolia* when injected intraperitoneally in mice resulted in the inhibition of lung metastatic colonies (Pingale 2010).

Antidiabetic activities:

Diabetes mellitus is a worldwide chronic disease of human related with the elevated blood sugar level due to insulin deficiency. Diabetes is broadly classified into type -1 and type 2-diabetes. *Tinospora cordifolia* has shown significant reduction in blood sugar level in both normal and alloxan induced

mice. The extract of plants parts decrease the blood sugar level (Chattopadhyay, 1999).

The root extract of *Tinospora cordifolia* decreased the level of glycosylated haemoglobin, hydroperoxidase and vitamin E (Umamasheswari and Prince, 2007). Grover *et al.*, studied the hypoglycaemic effect of aqueous extract of *Tinospora cordifolia* at different time intervals from 21-120 days in mice. At a dose of 400mg/kg / day the aqueous extract exhibits a significant (70.37 %) decrease in the plasma sugar level in mild diabetes (plasma sugar level > 180mg/dl, duration 21days) but in moderate diabetic condition hypoglycaemic effect is decreased to 48.61% and 0% in moderate diabetes ((plasma sugar level > 280mg/dl, duration 120 days) and severe diabetes (plasma sugar level > 4000mg/dl, in 120 days of duration) . In this study methanolic extract of *Tinospora cordifolia* showed anti- hyperglycaemic property in streptozotocin induced diabetic mail albino rats (250-400mg/dl of blood glucose level was taken for experiment) in treated rats gluconeogenesis had significantly decreased by less activity of enzyme glucose-6-phosphate. On the other hand the activity of glucokinase/hexokinase had increased. This indicated that glucose level in treated rats was lowered down by the methanolic extract of stem of *Tinospora cordifolia*. Sharma and Prajapati, (2015) reported that *Tinospora Cordifolia* not only maintains glycemic control like those conventional drugs, but possesses multiple target actions obliterating the complex diabetic pathology and remove apparent metabolic complications. It possesses multiple beneficial activities via several extrapancreatic and intrapancreatic mechanisms attributed to improve the pathological status of diabetes.

8. Immunostimulatory effects of *Tinospora cordifolia*:

Tinospora cordifolia is widely used in Ayurvedia to treat various infections and diseases. Constituents particularly the polysaccharides isolated from its stem have been reported to have immune modulating properties by modern scientific research as well. For example RR1 (1,4- alpha-D- glucan) a polysaccharide obtained from *Tinospora cordifolia* was examined for its effect on various types of lymphocytes such as NK cells and B cells. RR1 at 100 µg/ml concentration significantly increased the activation of NK cells, B cells and T cells. The significant activation of NK cells was also evident from results of the functional cytotoxic assay. RR1 treated lymphocytes showed higher percentage of killing over tumour cell lines compared to the untreated ones in a dose dependent manner (Nair *et al.*, 2004). In a continuing study RR1 also showed dose dependent induction of TNF- alpha synthesis from macrophages (Nair *et al.*, 2006) . In another study, it was observed that the aqueous extract of *Tinospora cordifolia* was effective in boosting phagocyte mediated immune response invitro The extract at a concentration of 5µ g/ml showed 20% increase in phagocytic ability of macrophages and increased antibody production against sheep red blood cells used as antigens (Ranjith *et al.*, 2008). This study revealed the multifaceted immuno modulatory potential of *Tinospora cordifolia*. Multifaceted immunomodulatory potential of *Tinospora cordifolia* has been reported by Upadhyaya *et al.*, (2011). In this study dose- dependent cytotoxicity in B16F10 mouse melanoma cell. After 72 hours of treatment with 100µg/ml of *Tinospora cordifolia*, more than 90%

cytotoxicity was observed and also the aqueous extract of *Tinospora cordifolia* showed boosting of phagocytic ability of macrophages, significant increase in nitric acid production by stimulation of splenocytes and macrophages at 1mg/kg dose of the extract. More and Pai, (2011) reported the effect of *Tinospora cordifolia* on macrophage activation as immunomodulatory effect. In this study, macrophage cell line J774A showed significant enhancement in secretion of lysozyme and lipopolysaccharides after treated with *Tinospora cordifolia* drug at different time intervals (24hrs and 48 hrs). This study revealed that *Tinospora cordifolia* has the potential to act as immunomodulator for activation of macrophages. Sharma *et al.*, (2012) evaluated a large variety of compounds which are responsible for immune modulatory and cytotoxic effects including 11- hydromuskatone, N- methyl-2- pyrrolidone, N- formylanionian, cordifolioside A, mangoflorine, tinocordioside and syringin.

Radio protective Thakur and Lavanga, (2012) reported the ameliorative effect of alcoholic and aqueous extract of *Tinospora cordifolia* at a dose of 100 and 200mg/kg/p.o for 15 days. Results of this study showed that *Tinospora cordifolia* attenuated sciatic nerve root ligation induced foot deformation, cold hyperalgesia, motor in- coordination, inflammation and reversed the ligation induced alterations in super-oxide dismutase (SOD), catalase (CAT), lipid peroxide and total calcium levels. The rats treated with alcoholic extract showed better activity than the rats aqueous extract of *Tinospora cordifolia*.

Another experiment showed that *Tinospora cordifolia* has the potential to alleviate the radiation mediated adverse effects and it could be exploited as a protector against planned and unplanned radiation exposure. Results of this experiment revealed that irradiation of mice caused a considerable elevation in glycogen, total proteins, acid phosphates and LPO along with a significant decrease in alkaline phosphate, GH, Catalase and SOD activities. On the contrary, oral administration of *Tinospora cordifolia* extract before irradiation reduced the radiation -induced variations in all such parameters and the recovery and regeneration was faster as compared to irradiated control group (Priyanka and Pradeep Goyal., 2014).

9. Antimicrobial activities:

Tinospora cordifolia extract has been reported against bacterial growth and improved phagocytic and intra cellular bacterial capacities of neutrophils in mice (Sengupta *et al.*, 2009). *Tinospora cordifolia* compounds showed promising antibacterial and antifungal activities against all tested organisms. The minimum inhibitory concentration of *Tinospora cordifolia* compounds against *Bacillus megaterium* and *Salmonella typhi* -A was found to be 128 µ/ml in nutrient broth medium. The value of nutrient lethal concentration, Lc50 (9.3 µ/ml) indicated the high toxic effect of *Tinospora cordifolia* compound . In vitro antibacterial activity of hot and cold methanol stem extract of *Tinospora cordifolia* was performed by cup plate agar diffusion method using ciprofloxacin as a standard drug for comparing antibacterial activity. Maximum anti bacterial activity of hot and cold methanol extracts was shown against *Staphylococcus aureus* when

compared with standard drug ciprofloxin. In another recent study, antimicrobial activity and phytochemical screening of *Tinospora cordifolia* and *Euphorbia hirta* is done. The results of this experiment showed that the diameter of inhibition decreased with concentration of plant extract. The leaves extract of the plant species under study were found to contain tanins, cardiac glycosides, terpenoids, carbohydrates and sponin. All these crude extracts has shown zone of inhibition against *Serratiamar cescens*, *Fusariumoxy sporium*, *Trichoderma reesei*, *Aspergillus niger*, *E.coli* at all concentrations (Sandhuet *et al.*, 2013). Same study was carried out by Hossain. *et al.* (2013) in which antimicrobial activity of *Tinospora cordifolia* extract by using different extraction solvents was investigated against two Gram positive bacteria (*Bacillus subtilis* and *Sarcinalutea*) and two Gram negative bacterias (*E.coli* and *Klebsiellap neumoniae*). All the solvent extract showed susceptibility against three above bacteria where as *Klebsiellap neumoniae* showed resistance.

10. Role in pharamaceutical science:

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