CHAPTER 1

INTRODUCTION

India is a rich repository of higher plants being a home for over 45,000 different plant species. It is one of the world’s 12 Biodiversity Centers with 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (Joy et al. 1998). Estimates suggest that about 40% of the higher plant diversity of the country is used in its codified and folk healthcare traditions (Ved and Goraya, 2007). Plant based drugs constitute about 80% of the total drugs in fast developing countries such as China and India, whereas, this share is only 25% in developed countries such as United States of America. Therefore, medicinal plants are much more economically important for countries like India than to rest of the world. It is therefore, necessary to select, characterize, multiply and conserve genetically superior planting material of important medicinal plants for assured uniformity and desired quality. The Task Force on Conservation and Sustainable Use of Medicinal Plants in India has identified Tinospora species as one of the most commercially exploited plants for pharmaceutical applications. The estimated annual demand of this species used in the preparation of crude herbal drugs in the Indian system of medicines is 10,000 tones (Singh and Warrier, 2004). Tinospora cordifolia is categorized as “Rasayana” in traditional Indian System of Medicine “Ayurveda” and is used as general tonic because of its anti-inflammatory, anti-arthritic, anti-allergic, anti-malarial and immunomodulatory properties (Chopra et al. 1956; Kirtikar and Basu, 1975; Nadkarni and Nadkarni, 1976; Chopra et al. 1982; Rawal et al. 2009). Commonly known as “Amrita or Guduchi” is an important drug of Indian System of Medicine (ISM) and used in medicines since time immemorial. The drug is well known Indian bitter and stipulate in fevers, diabetes, urinary problems and skin disease. “Guduchi satva” the starch obtained from the stem is highly nutritive and used to treat many diseases.

Guduchi, as it is most commonly called, has been described as “One Which Protects the Body”. The Sanskrit and Hindi name Amrita is derived from ancient Hindu scriptures where Amrita was used to bring the dead back to life and keep Gods from growing ill and old. It is no wonder that it is also referred to as “Nectar of Immortality” and “Heavenly Elixir”. Common name of Tinospora cordifolia include: Guduchi, amrita (Sanskrit), giloya, amrita (Hindi), giloe, gulancha (Bengali), gado, galo (Gujarati), duyutige, teppatige (Telugu).
heartleaf moonseed, Tinospora (English) (Nadkarni and Nadkarni, 1976; Pandey, 2002). In Hindi, the plant is commonly known as Giloyā, which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young (Singh et al. 2003; Panchabhāi et al. 2008; Kaplesh and Mohan, 2009).

**The family Menispermaceae**

Family Menispermaceae consists of about 70 genera and 450 species that are found in tropical lowland regions. These are generally climbing or twining, rarely shrubs. Leaves are alternate or lobed, flowers small cymose, seeds usually hooked or reniform. This family is rich source of alkaloid and terpenes (Sharma et al. 2010).

**The genus *Tinospora***

*Tinospora* is one of the most important genera of family Menispermaceae, consisting of about 15 species. Some medicinally important species includes *T.cordifolia, T.malabarica, T.tementosa, T.crispa, T.uliginosa* etc (Sharma et al. 2010).

**Botanical Description**

*Tinospora cordifolia* (Willd.) Miers ex Hook.F. and Thoms is a large, glabrous, dioecious, deciduous climbing shrub with several twining branches. The plant can be seen in most tropical and sub-tropical regions in India. The thickness of the stem is generally about 1 cm in diameter but sometimes it can be as thick as 6 cm. The leaves are heart shaped and the colour of the stem is white to grey. The stems of *Tinospora cordifolia* are 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 (Kirtikar and Basu, 1975). 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 pea sized. The seeds are curved. Fruits are fleshy and single seeded. Flowers grow during the summer and fruits during the winter (Singh et al. 2003). Figure 1.1 represents different stages of growth and development of *Tinospora cordifolia* - (A) Before Anthesis; (B) Emergence of flower; (C) Immature green fruits; (D) Cluster of mature red fruits
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Characterization of *Tinospora cordifolia* (Willd.) Miers germplasm

Figure 1.1 Different stages of growth and development of *Tinospora cordifolia* -
(A) Before Anthesis; (B) Emergence of flower; (C) Immature green fruits; (D) Cluster of mature red fruits
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Distribution

It is distributed throughout tropical Indian subcontinent including China, Burma and Srilanka, ascending to an altitude of 300 m. There is evidence that the plant is found in tropics of Africa and Australia (Singh et al. 1984). Its habitat ranges across a wide region in India spreading from Kumaon Hills to Kanyakumari, the Southern tip of India (Singh et al. 2003; Panchabhai et al. 2008; Kaplesh and Mohan, 2009). Three species of Tinospora including T. cordifolia Miers, T. malabarica Miers and T. crispa Miers. are found in India (Chadha, 1976). T. cordifolia is widely distributed throughout tropical regions but is abundant in the Northwestern region including Jammu & Kashmir and Himachal Pradesh. The other two species viz T. malabarica and T. crispa are found in limited areas. T. malabarica is found down in the south Kerala region of the country and T. crispa in the Assam region of Northeastern Himalayas (Ahmed et al. 2006 b). However, in some studies T. sinensis (Lour.) Merrill has been used as a synonym for T. malabarica Miers ex Hook. F. and T. crispa (Linn.) Miers ex Hook. F. & Thoms (Singh et al. 2004). Figure 1.2 represents Giloy climbing on Accacia nilotica and Bombax ceiba.

Systematic Position of T. cordifolia

The taxonomic hierarchy of the species Tinospora cordifolia is as follows:

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>- Planate</th>
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<tbody>
<tr>
<td>Phylum</td>
<td>- Magnoliophyta</td>
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<tr>
<td>Class</td>
<td>- Magnoliopsida</td>
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<tr>
<td>Family</td>
<td>- Meninspermaceae</td>
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<tr>
<td>Genus</td>
<td>- Tinospora</td>
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<tr>
<td>Species</td>
<td>- Cordifolia</td>
</tr>
<tr>
<td>Botanical name</td>
<td>- Tinospora cordifolia (Willd.) Miers</td>
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<tr>
<td>Synonyms</td>
<td>- Gulanshe Tinospara, Gulancha, Tinospara, Giloy</td>
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Figure 1.2 (A) Giloy climbing on Accacia nilotica (B) Giloy climbing on Bombax ceiba
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Multifaceted pharmaceutical applications of *Tinospora*:

As an Ayurvedic drug Guduchi or Guduchika has been described in various Ayurvedic texts such as Charaka Samhita, Sushruta Samhita, Ashtang Hridaya, Bhava Prakasha and Dhanvantari Nighantu. According to these treaties, Guduchi is useful in some of the ailments such as leprosy, mahajvara, asthma, anorexia, jaundice, gout, skin infections, diabetes, diarrhoea and dyspepsia.

- **Cancer control:** Cancer killing property has been observed in pre-clinical studies on Guduchi extracts. When cancer cells were exposed to the extracts of Guduchi, their numbers dwindled significantly. Thus, opening up a possible avenue in cancer control. Further studies have to be undertaken to isolate those compounds which have actually controlled the cancer growth.

- **Diabetes control:** When the extracts from Guduchi were fed to diabetes induced rabbits and rats, their blood glucose levels had decreased to a significant level, particularly the fasting blood sugar levels were controlled, implying a change in the metabolism of carbohydrates.

- **Cholesterol control:** Cholesterol control was also observed with the extracts of Guduchi roots. Administration of Guduchi root extracts on diabetic rats resulted in noticeable control of serum and tissue cholesterol.

- **Ulcer Treatment:** Ulcers can be a result of excessive acid secretion in stomach which damages the stomach walls. Generally, the stomach wall lining is affected regularly but, the stomach makes a new wall lining. But, if the stomach is not able to fully heal the wall lining, then the result is formation of ulcers. These ulcers have been controlled to an appreciable level by Guduchi extracts.

- **Liver protection:** For hundreds of years, Ayurveda has been prescribing Guduchi for liver disorders. Tests have shown that Guduchi speeds up regeneration of damaged liver tissue. Apart from extracts of Guduchi, a standard Ayurvedic medicine made from Guduchi was also tested which proved to be very effective on controlling liver damage.

- **Post-menopausal syndrome releives:** Guduchi has been tested in women suffering with post-menopausal syndrome which can have symptoms such as breast discomfort, nausea and fluid retention. These symptoms and ultimately PMS were statistically reduced by administering Guduchi extracts.
A perusal of literature reveals that despite manifold medicinal applications and wide geographic distribution, studies based on advanced techniques including determination of nature and extent of variability in this pharmaceutically important alkaloid Berberine is very limited. *T. cordifolia* has become an imperative issue of research due to the occurrence of abundant pharmaceutically important bioactive agents. Although no other natural product is as effective as berberine against treatment of multiple ailments including anti-cholesterol and anti-diabetes lowering activity but there is a need to evaluate the complexity of different bioactive components present in the herbal concoction by rigorous assessment of the mechanism of drug action, pharmacokinetics, metabolism, effective concentration, ratio of impurities, bioavailability and bibliographic data which will reveal the safety and efficacy of the drug after a long-term experience. Therefore, cost effective and easily available drug delivery systems based on these alkaloids having no toxicity can be established on a large scale. Molecular mechanisms underlying berberine action must be elucidated precisely so that various pharmaceutical applications of Berberine including anti-cancerous and diabetes-protective properties can be used for saving thousands of lives annually.

Although this plant has many excellent traits, adequate attention has not been focused on its conservation and genetic improvement. Conventional propagation methods i.e., seeds and cuttings are in place but have many limitations. Propagation can be done vegetative by cuttings, but this is season dependent. Biotechnological approaches such as cell and tissue culture can be a practical solution to these problems. Therefore, application of contemporary biotechnological implementations needs to be optimized for harnessing maximum benefits from this pharmaceutically important plant. An efficient regeneration system needs to be in place for improvement of this genus through genetic transformation and production of useful metabolites in cell cultures. Studies are in progress for micropropagation through shoot multiplication and somatic embryogenesis, as well as for secondary metabolite (berberine) production in callus cultures and bioreactors. Moreover, little information is available regarding the genetic variability through application of molecular markers. The Biotechnological tools can play an important role in identification, selection, multiplication and conservation of elite genotype the critical genotypes. *In vitro* regeneration holds tremendous potential for the production of elite genotypes of this species. Further, biotechnological tools and techniques offer scope for multiplication and genetic enhancement of desirable genotypes and the improved *in vitro* plant cell culture systems have potential for commercial exploitation of secondary metabolites.
Keeping these issues in the background and to understand the genetic diversity among giloy cultivars in a systemic way with identification of high Berberine yielding ecotypes the present investigation entitled “Characterization of *Tinospora cordifolia* (Willd.) Miers germplasm using molecular marker and berberine content” was undertaken with following objectives:

- **Collection of germplasm from indigenous sources**
- **Estimation of Berberine content in *Tinospora cordifolia* in collected germplasm**
- **Characterization of the available germplasm on the basis of Berberine content**
- **Identification of elite lines on the basis of Berberine content**
- **Optimization of conditions for DNA fingerprinting using RAPD and ISSR markers**
- **Assessment of genetic diversity and relatedness among *Tinospora cordifolia***