CHAPTER I

1. INTRODUCTION

1.1 Medicinal plants

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization estimates that up to 80 percent of people still rely mainly on traditional remedies such as herbs for their medicines. Plants are also the source of many modern medicines. It is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from or modeled on plant substances.

Medicinal plants are the most important source of life saving drugs for the majority of the world’s population. Medicinal plant research continues to explore Indian traditional medicines to develop the novel drugs. There is a great demand for herbal medicines in both developed and developing countries because of their wide range of biological activities, without causing side effects, than the synthetic drugs and lesser costs.

1.2 Conservation of medicinal plants

India is one of the twelve megadiversity countries of the world with a rich diversity of biotic resources (Bapat et al., 2008). A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category. India has a rich biological diversity due to its varied climatic, altitudinal variations and ecological habitats. There have been increasing rates of threats of depletion to these biological resources due to immense biotic and abiotic stresses. Indiscriminate collection of plants for their medicinal, ornamental, perfumery uses etc., and habitat loss and degradation are potential causes of threats.

Medicinal plants are an integral component of research and development in the pharmaceutical industry. They constitute nearly 70% of the basis of modern pharmaceutical products including 25% of drugs derived from different plants and
many others are synthetic analogues built on prototype compounds isolated from them (Malik et al., 2012). The use of herbal medicines is growing in developed countries, presently 25% of the world population use herbal medicine (Zhou et al., 2006). About 40% of compounds used in pharmaceutical industry are directly or indirectly derived from plants because the chemical synthesis of such compounds is either not possible and/or economically not viable (Rout et al., 2000). Therefore, a large number plant species (especially medicinal) are under threat of extinction because of their over exploitation. In last two decades there has been a great increase in research on medicinal plant. A number of new medicines have been discovered and advancements in production technology to harvest pharmaceutical important metabolites.

The basic principle governing the conservation of any species is the inclusion and maintenance of maximum genetic diversity. Incidentally, genetic diversity in plant populations is structured in a way that it reflects the biological characteristics, distribution and ecology of the species examined. Conventionally, there are two methods of conservation: in situ and ex situ conservation, both are complementary to each other. In situ methods allow conservation to occur with ongoing natural evolutionary processes, ex situ conservation via in vitro propagation also acts as a viable alternative for increase and conservation of populations of existing bioresources in the wild and to meet the commercial requirements (Kapai et al., 2010).

1.3 The biotechnology tool

Biotechnological tools are important for multiplication and genetic enhancement of the medicinal plants by adopting techniques such as in vitro regeneration and genetic transformation. However most of the plants used as medicines in traditional practice are rare and not found commonly. Over exploitation of the natural herbs would soon result in depletion of the wild species and they would become extinct. Also, export potential of the medicinal herb is increasing, resulting in commercial cultivation of the medicinal herbs. The modern biotechnological tools help in the production of large number of plants by tissue
culture. Many government and private agencies produce herbs with good qualities for supply to farmers for commercial cultivation (Leena and Jaindra, 2003).

Biotechnology offers an opportunity to exploit the cell, tissue, organ or entire organism by growing them in vitro and to genetically manipulate them to get the desired compounds. Since world population is increasing rapidly, there is extreme pressure on the available cultivable land to produce food and fulfill the needs. Therefore, for other uses such as production of pharmaceuticals and chemicals from plants, the available land should be used effectively.

Tissue culture is one of the techniques in biotechnology, which has brought about significant impact in the field of plant breeding and conservation of many endangered plants. At present, the unique regeneration properties of plants and their biochemical potential have been exploited fully. Molecular investigations coupled with breeding and genetic engineering techniques are being pursued to improve their nutritive and medicinal components in medicinal plants for higher productivity.

1.4 Plant tissue culture

Tissue culture, an important area of biotechnology can be used to improve the productivity of planting material through enhanced availability of identified planting stock with desired traits (Baskaran and Jayabalan, 2005). Advanced biotechnological methods such as plant cell, tissue and organ culture are playing a major role in production of valuable secondary metabolites as well as propagation of important medicinal plant species. In vitro propagation is a rapid method for production of true-to-type medicinal plants. Many medicinal plant species are disappearing at an alarming rate, as a result of rapid agricultural and urban development, deforestation and indiscriminate collection. Plant tissue culture technology may help to conserve rare and endangered medicinal plants. During the last decade in vitro propagation of medicinal plants has made significant contribution to the pharmaceutical industry. It is hoped that in vitro propagation of medicinal plants would become a multi-dollar industry in the years to come.

One of the main applications of in vitro propagation is the mass propagation of superior plants. In many instances, conventional propagation is a slow process
during which diseases and pest problem can limit production. In vitro propagation offers the potential to produce thousands or even billions of plants per year. In vitro propagation allows the production of large of plants from small pieces of stock plant in relatively short period. Depending on the species, the original tissue explant is taken from shoot tip, leaf, lateral bud stem or root tissue. Once the explant was placed on an appropriate culture medium, proliferation of buds and adventitious shoots results in tremendous increase in the number of shoots. In vitro techniques have been routinely adapted for the propagation and effective multiplication of many medicinal plants to meet the demand of pharmaceutical firms and to protect the natural population of rare and endangered plant species. The ability to regenerate whole plants from somatic tissues through organogenesis is an added advantage under in vitro propagation and also it is a prerequisite for genetic transformation of plants.

As medicinal plants are constantly under threat because of over exploitation and depletion of natural habitats the need for their depletion rapid multiplication and conservation have gained importance. During the past twenty years tremendous progress has been made in the area of species conservation that directly relates to the development of new, simple and effective techniques.

1.5 DNA fingerprinting analysis

Molecular systematic study is essential in medicinal plants and supported by phytochemical studies, it will be helpful to understand the nature of distribution and variability among them which is important for the identification and selection of superior genotypes and for further exploitation. In recent times there is an increased emphasis in molecular markers for characterization of genotype, genetic variability, genetic fingerprinting, for identification and cloning of important genes marker assisted selection and in understanding of inter relationship at molecular level.

DNA based molecular markers are efficient tools for identification of genetic variation, cultivars, disease resistance genes and authentication of medicinal plant species. This technique remains important in plant genome research with its applications in pharmacognostic identification and analysis. In India, few research
institutes have started experiments on exploring DNA-based techniques for genotyping of medicinal plants. RAPD technique has been widely used to construct genetic linkages of medicinal plant species and RFLP technique has also been applied for the characterization of medicinal plants species at genetic level. In the present study, DNA fingerprinting analysis was used to determine the genetic relationships among the selected medicinal plant species.

RAPD markers are a modification of PCR contrived in the late 1980’s (Williams et al., 1990). PCR provides a mean by which billions of copies of a particular target DNA fragment can be made from a complex mixture of genomic DNA. Now it is becoming more powerful with the introduction of user-friendly and fully automated techniques. RAPD method is relatively quick when compared to other markers, reveals greater genetic variability due to the regions in which amplification takes place and useful in differentiating closely related individuals (Manimekalai et al., 2007).

1.6 Phytochemical diversity

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Medicinal plants possess unlimited and untapped wealth of chemical compounds with high drug potential which make these plants useful as sources of biomedicines (Oladunmoye et al., 2009). Natural products play a dominant role in the development of novel drug for the treatment and prevention of diseases (Gilani and Rahman, 2005). Interestingly, it is estimated that more than 25% of the modern medicines are directly or indirectly derived from plants. Ethnopharmacological studies on such herbs/medicinally important plants are an area of interest for the investigators throughout the world.

It is noteworthy that genetic and environmental factors and their interactions affect the pharmaceutically important secondary metabolites in medicinal plants. A variety of environmental factors, such as season, altitude, radiation and soil nutrition, have been proven to significantly influence the secondary metabolite
profile. Quantitative evaluation of phytochemical diversity in medicinal plant species population from different natural geographic areas supports the existence of distinct natural chemotypes within the species.

The difference in chemical components in medicinal plants will affect the quality of pharmaceutical products and the standardization of herbal medicine. Furthermore, the safety and quality of raw materials from a medicinal plant depend significantly on its intrinsic and extrinsic factors. As WHO has defined, the intrinsic factors include genetic influences and extrinsic factors include environmental conditions, collection methods, cultivation, harvest and post-harvest processing, transportation and storage practices (WHO, 2003). Chromatographic fingerprinting analysis is an easy method to evaluate a sample without sufficient information of chemical components, if one or two reference components are known. This method has been accepted as a strategy for quality assessment of herbal medicines by the WHO (2000), the FDA (2000), the European Medicines Agency (EMA) (2001), the Chinese, State Food and Drug Administration (SFDA) (2000) and other authorities. To date, chromatographic fingerprinting analysis has been reported for many herbal medicines.

1.7 Nanobiotechnology

Nanobiotechnology is a new frontier for biology with important applications in medicine. It bridge areas in physics, chemistry and biology and is a testament to the new areas of interdisciplinary science that are becoming dominant in the twenty-first century. Nanomedicine, a new attractive term frequently applied nowadays that implies for the medical application of nanotechnology as an alternative to the classical drug formulations. In the last decade, an increasing number of investigations concerning the use of nanoscale structures for drug and gene delivery purposes have been developed (Jin and Ye, 2007). Despite the significant scientific interests and promising potential in numerous applications, the safety aspects of nanoparticulate systems remain a growing concern as the processing of nanoparticles in biological systems could lead to unpredictable effects. In addition, due to the greater surface area-to-volume ratio for nanoscale material, the toxicity could differ from a similar bulk material (Xia et al., 2006).
Most of the chemical methods used for the synthesis of nanoparticles are too expensive and also involve the use of toxic, hazardous chemicals that are responsible for various biological risks. This enhances the growing need to develop environment friendly processes through green synthesis and other biological approaches. The synthesis of nanoparticles using various plants and their extracts can be advantageous over other biological synthesis processes which involve the very complex procedures of maintaining microbial cultures.

1.8 Diabetes mellitus

Diabetes mellitus is a complex and a multifarious group of disorder that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin. Diabetes mellitus is also one of the most common chronic diseases across the world and number of diabetic patients is on rise. In 2011, there were 366 million people with diabetes globally, and this are expected to rise to 552 million by 2030. Most people with diabetes live in low and middle-income countries like India and these countries will also see the greatest increase over the next 19 years. The recently published ICMR-INDIAB national study reported that there are 62.4 million people with type 2 diabetes (T2DM) and 77 million people with pre-diabetes in India. These numbers are projected to increase to 101 million by the year 2030 (Whiting et al., 2011). This surprising increase was primarily rooted in genetic level and modern life style related factors like obesity, physical inactivity, aging, nutrition and stress.

1.8.1 Antidiabetic activity of medicinal plants

The hyperglycemic condition of a diabetic patient is untreatable but the blood glucose level can be controlled by daily dose of antidiabetic drugs. A number of FDA approved antidiabetic drugs are chemically synthesized like sulfonylureas, biguanides (α-glucosidase/starch inhibitors), thiazolidinediones (insulin secretagogues), natural extracts products (genistein, astrilbin, hesperidin) and rDNA technology (insulin). These drugs were being used for the control of blood glucose level in diabetic patient. However, recently preferences are given to naturally formulate antidiabetic compositions due to their less toxicity and negligible side
effect to the body. A number of natural extracts from medicinal plants were in use since ancient time to control the diabetes but recently research for new and more effective natural antidiabetic drugs are in progress (Jung et al., 2006).

Figure 1. Schematic representation of mechanism and action of some antidiabetic medicinal plants

1.9 Importance of the study

Medicinal plants are of great interest to researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Chand et al., 1997). Diabetes mellitus is a common disorder among the Indian population. The management of diabetes is a global problem until now and successful treatment is yet to be discovered. There are many synthetic medicines/drugs developed for patients, but it is the fact that it has never been reported that someone had recovered completely from diabetes (Li et al., 2004). The modern oral hypoglycemic agents produce undesirable and side effects. Thus, alternative therapy is required to shift towards the different indigenous plant and herbal formulations (Satyanarayana et al., 2007). Plant drugs are frequently considered to be less toxic and free from side effects than synthetic one. With the
worldwide increasing demand for plant derived medicines, there has been a
concomitant increase in the demand for raw material. However, the increasing
human and livestock populations affected the status of wild plants, particularly those
used in herbal medicine.

Several medicinal plants were mentioned in the indigenous medicinal system
to cure of diabetes and some of them have been experimentally evaluated and active
compounds were also isolated. The ethnobotanical information report states that
about 800 plants may possess antidiabetic potential. Recently, the medicinal values
of various plant extracts have been studied by many scientists in the field of diabetic
research. Among them, Gymnema sylvestre and Stevia rebaudiana are having
excellent antidiabetic properties and became endangered at present due to its
overexploitation.

The advantages in molecular biological research have given a new dimension
to in vitro culture as well as for plant improvement; enhancing the yields of the
product and resulting in multiple products or producing novel products form
genetically engineered plants. Moreover, the need for safer drugs without side
effects has led to the use of natural ingredients with proven safety. These factors
have laid emphasis on the use of biotechnological methods to enhance the
production of pharmaceuticals and food additives in quality and quantity.

Nanobiotechnology is a promising field especially for biodiversity rich
countries like India. Biological diversity can be thus used as a major resource for
biotechnological products and processes, which may be suitable for large scale
synthesis. Green procedures for nanoparticle synthesis may be suitable for diverse
applications that are continuously being explored. Design and development of
biodegradable controlled drug delivery of therapeutic entities with improved
bioavailability is the main research aspect on which extensive work has been done in
the past few decades. The promising and exciting drug delivery system which can be
met the above mentioned requirements is polymeric nanoparticles. Chitosan is the
abundant biodegradable natural polymer with great potential for pharmaceutical
applications due to its biocompatibility, high charge density, non-toxicity and
mucoadhesion. It has been reported that high molecular weight chitosan

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demonstrates the potential of reducing hyperglycemia and hypercholesterimia in streptozotocin induced diabetic rats. Chitosan-based nanoparticles are simultaneously, too short, the very important carriers of drug delivery and the supplement of antidiabetic drugs. Therefore, it is feasible to develop chitosan nanoparticles as the carrier for exploring effective mechanism of plant based antidiabetic drugs.

1.10 Plants description

1.10.1 Gymnema sylvestre R.Br.

Gymnema sylvestre (GS) is a slow growing, perennial, woody climbing plant (Asclepiadaceae family), which grows in tropical forests of central and southern India. Leaves are opposite, usually elliptic or ovate (1.25–2.0 inch × 0.5–1.25 inch); flowers are small, yellow, in umbellate cymes.

1.10.1.1 Scientific classification

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Botanical Name: Gymnema sylvestre R.Br. Figure 2. Gymnema sylvestre plant

Sanskrit/Indian name: Meshashringi, Vishani, Madhunashini, Gurmar, Merasingi

1.10.1.2 Medicinal uses

Gymnema sylvestre is a herb native to the tropical forests of southern and central India and Sri Lanka. Chewing the leaves suppresses the sensation of sweet, hence the popular Hindi name gurmar (sugar destroyer). This effect is attributed to the eponymous gymnemic acids. G. sylvestre has been used in herbal medicine as a treatment for diabetes for nearly two millennia, and though there is insufficient scientific evidence to draw definitive conclusions about its efficacy two small clinical trials have shown Gymnema to reduce glycosylated hemoglobin levels (Yeh et al., 2003).
Ayurveda as well as modern research together highlighted the medicinal use of G. sylvestre as potential antidiabetic agent. Besides this, in prevailing systems of medicine, the plant is also used in the treatment of jaundice, haemorrhoids, renal and vesicle calculi (Saneja et al., 2010), cardiopathy anti-hyperglycemic (Shanmugasundaram et al., 1990), anti-hyperlipidemic (Rachh et al., 2010), antimicrobial (Chodisetti et al., 2013), anti-oxidant (Kang et al., 2012), anti-inflammatory (Saneja et al., 2010) and anti-cancer (Srikanth et al., 2010) activities. Leaf extracts was also found to be effective in reducing elevated serum triglycerides, total cholesterol and very low density lipoprotein and low density lipoprotein (Rachh et al., 2010). Beside these activities, the plant has also shown antimicrobial (Pasha et al., 2009; Satdive et al., 2003), anti-inflammatory (Malik et al., 2008) and free radical scavenging (Ohmori et al., 2005) activities. Gymnemic acid, a group of complex triterpenic glycosides was reported to be responsible for the antidiabetic action. Gymnemagenin, a common aglycone of gymnemic acids produced after acidic and basic hydrolysis, has been shown to inhibit glucose absorption and has been commonly used by several workers as an analytical marker to determine the quality of Gymnema plant materials (Puratchimani and Jha, 2004).

1.10.2 Stevia rebaudiana Bertoni

Stevia is a genus of about 200 species of herbs and shrubs in the sunflower family (Asteraceae), commonly known as sweet leaf, sugar leaf, which is widely grown for its sweet leaves, the source of sweetener products known as Stevia. As a sweetener and sugar substitute, stevia's taste has a slower onset and longer duration than that of sugar and some of its extracts may have a bitter or licorice-like after taste at high concentrations. With its steviol glycoside extracts having up to 300 times the sweetness of sugar; Stevia has attracted attention with the rise in demand for low-carbohydrate, low-sugar sweeteners. Because stevia has a negligible effect on blood glucose it is attractive to people on carbohydrate-controlled diets.
1.10.2.1 Scientific classification

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Figure 3. Stevia rebaudiana plant

1.10.2.2 Botanical description

It grows up to 1 m tall (Mishra et al., 2010). The plant is a perennial herb with an extensive root system and brittle stems producing small, elliptic leaves (Shock, 1982). The leaves are sessile, 3–4 cm long, spatulate shaped with blunt-tipped lamina, serrate margin from the middle to the tip and entire below. The upper surface of the leaf is slightly granular pubescent. The stem is woody and weak-pubescent at the bottom. The rhizome has slightly branching roots. The flowers are pentamers, small and white with a pale purple throat. Among the 230 species in the genus Stevia, only the species rebaudiana and phlebophylla produce steviol glycosides (Brandle and Telmer, 2007).

1.10.2.3 Medicinal uses

Stevia rebaudiana leaves contain non cariogenic and non-caloric sweeteners (steviol glycosides) whose consumption could exert beneficial effects on human health (Gardana et al., 2010). Stevia glycosides possess valuable biological properties. Regular consumption of these compounds decreases the content of sugar, radionuclides and cholesterol in the blood (Atteh et al., 2008), improves cell regeneration and blood coagulation, suppresses neoplastic growth and strengthens blood vessels (Jeppesen et al., 2003; Barriocanal et al., 2008). They also exhibit choleretic (Kochikyan et al., 2006), anti-inflammatory (Jayaraman et al., 2008; Sehar et al., 2008) and diuretic properties; they prevent ulceration in the gastrointestinal tract (Kochikyan et al., 2006), including antihypertensive (Jeppesen et al., 2002), antihyperglycemic (Chen et al., 2006); anti human rota-virus activities.
(Takahashi et al., 2001), glucose metabolism (Suanarunsawat and Chaiyabutr, 1997) and renal function (Jutabha et al., 2000). They present potential applications as antidiarrhoeal therapeutics (Chatsudhipong and Muanprasat, 2009). In addition, the Stevia plant and stevioside have been used in the treatment of cancer and as substitutes for saccharose in the treatment of diabetes (Jeppesen et al., 2000; Chen et al., 2006; Pol et al., 2007), obesity and hypertension (Chan et al., 2000; Lee et al., 2001; Hsieh et al., 2003; Goyal et al., 2010). They can also act as an anti-cariogenic product and as antgingivitis (Slavutzky, 2010).

1.11 Objectives

The main objectives of the present study are;

To develop an efficient in vitro propagation system for large scale plant production from two endangered medicinal plant species.

To examine the genetic uniformity by DNA fingerprinting analysis.

To investigate the genetic and phytochemical diversity within the populations of selected medicinal plant species.

To synthesize biomolecules loaded chitosan nanoparticles from two medicinal plants and to evaluate its antidiabetic activities in experimental rat model.