Introduction
1. Introduction

The earth is home to a rich and diverse array of living organisms, whose genetic diversity and relationships with one another and with their physical environment constitutes biodiversity. Biological diversity is the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and their ecological complexes. This includes diversity within species, between species and of ecosystems (Kushwaha and Kumar, 1999). In this way, biodiversity includes variety of all forms along with their genetic make up and their all possible assemblages. This biodiversity is the natural biological capital of the earth, and its conservation and sustainable management presents important opportunities for all nations, especially India.

Biodiversity of plants collectively known as 'plant genetic resources' is a key component of any production system - indeed, of any ecosystem, without which, natural evolutionary adjustment of the system to the changing environmental and biotic conditions would be impossible. Plant biodiversity is an irreplaceable resource, providing raw materials for introduction, domestication as well as improvement programmes in agriculture and forestry. Conservation and use of genetic diversity for sustainable ecosystem or agro ecosystem should be continuous to meet food, clothing, shelter and health requirements of India's growing population (FAO, 2002).
Plants are an important source of medicine – they are key ingredients in many medicines from aspirin to contraceptive pills and are the foundation of health care systems all over the world. One of the major causes for the depletion of plant life is the excessive collection of plants for medicinal purposes. Considering the fact that not even $\frac{1}{5}$ of all the plant life in the world has been documented, the very loss of the known plant species valuable for human survival can have catastrophic effects in the future. Around 119 pure chemical substances extracted from some 90 species of higher plants are used in medicines throughout the world. In the regional, traditional medicine systems, a wide range of plant species are used medicinally. The World Health Organization recognizes that medicinal plants play an important role in the health care of about 80 percent of world population in developing countries and depend largely on traditional medicines, of which herbal medicines constitutes the most prominent part (Farnsworth et al., 1988). The rest of the 20 percent also depend substantially on the plant-based medicines. WHO (2001) has listed over 21,000 plant names that have reported medicinal uses around the world. Very few of these have been subjected to scientific scrutiny.

"Medicinal plants are nature's hidden and to a large extent unexplored treasure. Although they have been used as a source of safe and effective medicine since time immemorial, this potential resource has hardly been commercially tapped. India is one of the 12-mega biodiversity centres having about 10% of the world’s biodiversity wealth, which is distributed across 16 agro-climatic zones. Out of 17,000 species of higher plants reported to occur within India, 7500 are known to have medicinal uses (Singh, 2001). This proportion of medicinal plants is the highest known in any other country against the existing flora of that country (Kala et al., 2006).
Tamil Nadu is gifted with rich biodiversity. The main natural habitat types are Forest, Mountains, Rivers, Wetlands, Mangroves and Beaches. Tamil Nadu has a geographical area of 1,30,058 km, which constitutes about 4% of the country's total area. Tamil Nadu shares the Western Ghats (one of the 25 biodiversity hotspots) with the states of Kerala, Karnataka, Goa, Maharashtra and Gujarat. It shares the Eastern Ghats with the States of Andhra Pradesh and Orissa (Biological Diversity Act, 2002; Biological Diversity Rules, 2004). Tamil Nadu accounts for nearly 1/3 of the total flora of India. Species and Generic diversity of flora of Tamil Nadu is comparatively richer than the neighbourhood states (Draft Tamil Nadu State Forestry Action Programme, 1999).

India has several traditional medical systems, such as Ayurveda, Siddha and Unani, which has survived through more than 3000 years, mainly using plant-based drugs. Ayurveda is the oldest medical system in the Indian subcontinent, followed by the Siddha and Unani medical systems. The *Materia Medica* of these systems contains a rich heritage of indigenous herbal practices that have helped to sustain the health of most rural people of India. The ancient texts like Rig Veda (4500-1600 BC) and Atharvana Veda mention the use of several plants as medicine. The books on Ayurvedic medicine such as *Charaka Samhita* and *Susruta Samhita* refer to the use of more than 700 herbs (Jain, 1968). The Glossary of Indian Medicinal Plants has listed around 3,000 plants (Asolkar et al., 1992; Chopra et al., 2000). Two thousand five hundred plants have been reported to be used in ethno-medicine (Jain, 1991). The number of plants listed in Ayurvedic *Materia Nighantu* is 560 in Bhav Prakash Nighantu (Kumar, 2000). The Ayurvedic Drug Formulary prepared by Department of Indian System of Medicine, lists 387 plants (Sarin, 1996). The Unani System of Medicine describes 440 plants
(Said, 1969) out of which 360 are common to other systems practiced in the country. The number of plants having confirmed therapeutic properties or yielding a clinically useful chemical compound thus lies around 700 species.

From the trade data available, it is clear that the global market for medicinal plants has always been very large. To give an example of the extent of trade volumes even at that time, according to one report commissioned by the World Wide Fund for Nature, the total import in 1980 of “vegetable materials used in pharmacy” by the European Economic Community was 80,738 tons (Lewington, 1993). India is one of the largest suppliers by far, with 10,055 tons of plants and 14 tons of vegetable alkaloid and their derivatives (Deshpande et al., 2008). It is also true that due to the rising international demand, many important medicinal plant species are becoming scarce and some are facing the prospect of extinction. Therefore it is important to conserve the extensively traded medicinal plants in its natural environment or cultivating it in favorable environments.

Biotechnology in its broadest sense includes plant tissue culture, the use of molecular markers both for breeding and fingerprinting purposes; the use of molecular tools to study gene expression; as well as the use of all this information for genetic engineering of plants. Genetic fingerprinting could be a powerful tool in the field of medicinal plants, to be used, for example, for correct germplasm identification. In addition, when linked to emerging tools such as metabolomics and proteomics, which could be seen as a fingerprinting technique on the plant’s metabolites or protein composition, it cannot only give data on phenotypic variation, caused by growth
conditions or environmental factors, but also yield data on the genes involved in the biosynthesis (Fridman and Pichersky, 2005).

Population genetic methods are employed to analyze genetic variation observed in a wild species or in a collection of accessions of a crop (Wang et al., 2010). Genetic variation is generally distributed in a hierarchical way; i.e., within an individual, between individuals, within a population or a collection of accessions, between populations, within a region of origin, and between all populations and all regions inhabited by the respective species. The extent of genetic variation in a species and its distribution among populations (or other entities of subdivision) is determined by a large number of factors, including the mating system, the demographic history, the effective population size, and the extent of gene flow by migration or seed dispersal between populations. At each level, the genetic variants are united through interbreeding at some time point in the past. By analyzing the amount of variation and its partitioning over these hierarchical levels, we can draw important conclusions about the biology of a species or the domestication history of a crop.

Genetic Diversity

Genetic diversity is the sequence variation within species. Information on genetic diversity and relationships among and between individuals, populations, plant varieties, animal breeds and species is of importance to plant and animal breeders for the improvement of crop plants and animal breeds, for conservation biology and for studying the evolutionary ecology of populations. Genetic diversity studies can identify alleles that might affect the ability of the organism to survive in its existing habitat, or might enable
it to survive in more diverse habitats. This knowledge is valuable for germplasm conservation, individual, population, variety or breed identification (Duran et al., 2009).

A decline in genetic variation can undermine the ability of an organism to respond to natural selection and consequently limits its evolutionary potential. Small populations are often subject to the loss of alleles through genetic drift, or random fluctuations in allele frequency (Lande, 1988). Hence, genetic diversity measurements are important for selection of superior genotypes and for considering conservation of a particular species. This is one of the best methods to scrutinize the resources and identify the variants of economic value for conservation and utilization. DNA based molecular markers are proving to be a versatile tool in the plant genome analysis and in differentiating different genotypes. DNA markers are highly stable and specific. It has immense applications in the standardization of medicinal plants and its products.

Molecular Markers

Molecular markers are biochemical constituents (e.g. secondary metabolites in plants) and macromolecules, viz. proteins and deoxyribonucleic acids (DNA) that plays a very important role in Taxonomy, Physiology, Embryology, Plant breeding, Ecology, Genetic engineering etc. Analysis of secondary metabolites is restricted to those plants that produce a suitable range of metabolites which can be easily analyzed and which can be distinguished between varieties. Thus among all molecular markers DNA markers, are more suitable and ubiquitous to most of the living organisms (Agarwal et al., 2008; Solouki et al., 2008).
The analysis of genetic diversity and relatedness between or within different populations, species, and individuals is a central task for many disciplines of biological science. The classical strategies for the evaluation of genetic variability, such as comparative anatomy, morphology, embryology, and physiology, have increasingly been complemented by molecular techniques. Marker technology based on polymorphisms in proteins or DNA has catalyzed research in a variety of disciplines such as phylogeny, taxonomy, ecology, genetics, and plant and animal breeding. The following properties would generally be desirable for a molecular marker: moderately to highly polymorphic, codominant inheritance (which allows the discrimination of homo and heterozygous states in diploid organisms), unambiguous assignment of alleles, frequent occurrence in the genome, even distribution throughout the genome, selectively neutral behavior (i.e., no pleiotropic effects), easy access (i.e., by purchasing or fast procedures), easy and fast assay (e.g., by automated procedures), high reproducibility, easy exchange of data between laboratories, low cost for both marker development and assay (Weising et al., 2005; Farooq and Azam, 2002).

As the science of plant genetics progressed, researchers have tried to explore these molecular marker techniques for their applications in commercially important plants such as food crops, horticultural plants, etc. and recently in pharmacognostic characterization of herbal medicine. Cultivation of medicinal plants in a grower's field is a recent phenomenon. Industry prefers raw materials from cultivated source because of authentication, reliability and continuity. Non availability of quality planting material coupled with poor development and extension support in the cultivation and processing and also unorganized markets are the major constraints coming in the way of
commercialization of cultivation. Therefore, concentrated efforts are required, both in
collection and cultivation of medicinal plants, in order to ensure sustainability of the
industry.

Randomly Amplified Polymorphic DNA (RAPD)

Studies on genetic diversity within populations have been simplified by the
introduction of molecular analysis techniques. Various markers are available for DNA
finger printing analyses such as Amplified Fragment Length Polymorphism (AFLP),
Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSRs)
and Randomly Amplified Polymorphic DNAs (RAPD) (Misra et al., 2010; Agarwal
et al., 2008; Rahman et al., 2007; Zhang et al., 2007). Among these, RAPD is an
inexpensive and rapid method not requiring any information regarding the genome of the
plant, and has been widely used to ascertain the genetic diversity in several plants (Belaj
et al., 2001; Deshwall et al., 2005). RAPD analysis requires only a small amount of
genomic DNA and can produce high levels of polymorphism and may facilitate more
effective diversity analysis in plants (Hasan et al., 2009). RAPD analysis is a multilocus
arbitrary fingerprinting technique that can be used for determining genetic relationships
of various species (Ramshini et al., 2005; Hoque et al., 2005; Sadder 2006), as well as
determining the components of herbal medicinal mixtures (Shinde et al., 2007). In
addition, RAPD analyses are efficient, economical and tend to produce genetic markers
suited to the assessment of population, race and species-specific genetic variation
(Tripathi et al., 2009; Kumar et al., 2007). RAPD analysis provides information that can
help define the distinctiveness of species and phylogenetic relationships at molecular
level. Use of such techniques for germplasm characterization may facilitate the conservation and utilization of plant genetic resources, permitting the identification of unique genotypes or sources of genetically diverse genotypes. RAPD analysis has been used for genetic diversity assessment and for identifying germplasm in a number of plant species (Aswati et al., 2004; Kapteyn and Simon, 2002; Welsh and McClelland, 1990). Due to technical simplicity and speed, RAPD methodology has been used for diversity analysis in many medicinal plant species (Li et al., 2002; Padamalata and Prasad, 2006a, b and 2007).

The use of molecular markers in breeding is a widespread technique for commercial and economically important crops and medicinal plants. But for our knowledge concerning the genetic diversity analysis on Gymnema sylvestre, Tinospora cordifolia, Ocimum sanctum, Cassia angustifolia, Indigofera tinctoria and Centella asiatica are very limited.

**Gymnema sylvestre**

*Gymnema sylvestre* is regarded as one of the plants with potent anti diabetic properties. It is belongs to the family Asclepiadaceae. Leaves used as an antidiabetic agent. The plant is used as diuretic, antidiabetic, antibilious, emetic, expectorant, astringent and stomachic. (Chopra et al., 1969; Chopra et al., 1996; Khare, 2007).

**Tinospora cordifolia**

*Tinospora cordifolia* belongs to the family Menispermaceae. Due to the wide array of bioactive principles as well as proven medicinal uses of this plant, it has received
considerable scientific attention. Stem is an ingredient of several Ayurvedic preparations used in general debility, dyspepsia, chronic diarrhoeae, chronic dysentery, fevers and urinary disorders. It is used as tonic, antiperiodic and aphrodisiac. Leaf decoction is used in gout. Root is a powerful emetic and used for visceral obstruction; its watery extract is used in leprosy. Pulverized fruit is used as a tonic. (Yoganarasimhan, 2000; Chopra et al., 1996; Bhattacharjee, 1998; Khare, 2007).

**Ocimum sanctum**

The plant *Ocimum sanctum* belongs to family Lamiaceae. It is an important genus of aromatic herbs, under shrubs or shrubs. The plant is used medicinally in villages; it contains citric, tartaric and malic acids. The infusion of juice leaves is used to treat digestive complaints, bronchitis and catarrh and also administered locally to cure ring worm and skin diseases. A decoction of root is used in malarial fever. In Ayurveda and Siddha it is used in fever, coryza, mental disorders and disease of vatam, kapham, digestive disorders, cough, leucorrhea and ulcers (Chopra et al., 1969; Chopra et al., 1996; Yoganarasimhan, 2000; Khare, 2007).

**Cassia angustifolia**

*Cassia* is a large genus of over five hundred species and twenty four are native to India. The leaves and pods of *Cassia angustifolia* produce crude drug *Senna* and is often referred to as Indian Senna. Indian Senna is cultivated in Southern-Western arid tracts of Tamil Nadu in marginal lands. Senna leaf and pod is usually used in the form of infusion. It is also used as anthelmintic for intestinal worms and as a liver stimulant, febrifuge,
splenic enlargements, jaundice, amoebic dysentery and for skin diseases (Chopra et al., 1996; Bhattacharjee, 1998; Khare, 2007).

**Indigofera tinctoria**

The plant *Indigofera tinctoria* belongs to family Fabaceae. The extract of the plant is given to epilepsy and nervous disorders, used in bronchitis and as ointment in sores, old ulcers and haemorrhoids. The root of this plant used in hepatitis and scorpion sting. Root and stem is laxative, expectorant, febrifuge, anticephalalgic, anti-tumour, anthelmintic, promote growth of hair (Chopra et al., 1996; Khare, 2007).

**Centella asiatica**

*Centella* or Indian Pennywort, *Centella asiatica* (L.) Urban syn. *Hydrocotyle asiatica* L. belongs to the family Apiaceae. The leaves are used as brain tonic. The drug has weak sedative but cardio-depressent and hypotensive action (Chopra et al., 1996; Bhattacharjee, 1998; Khare, 2007). In Chinese medicine the herb is used for dysentry and summer diarrohea, vomiting, jaundice, urinary calculi, epistaxis and scabies (Physician Desk Reference for Herbal Drugs, 2000). In Homeopathic medicine it is used for skin diseases associated with itching and swelling. It is used in inflammation and ulceration of uterus, eczema, elephantiasis, ascariasis and in granular cervicites (Physician Desk Reference for Herbal Drugs, 2000; Singh and Rastogi, 1969).

There are thousands of medicinal plants which deserve comprehensive scientific standardization and evaluation. Knowing the superiority of a species is crucial and useful for research and development purpose, consumption purpose, commercial purpose and
sometimes for the plant identification purpose. The following medicinal species *Gymnema sylvestre* (Retz.) R.Br., *Tinospora cordifolia* (Willd.) Miers ex Hook. F. & Thoms., *Ocimum sanctum* L., *Cassia angustifolia* Vahl., *Indigofera tinctoria* L. and *Centella asiatica* (L.) Urban. are distributed in various parts of Tamil Nadu. These are also cultivated for export to many countries. There is a gap between supply and demand. At the same time, because of many factors such as climatic, edaphic and methods of cultivation, batch-to-batch variation has been reported in all these species. Variability within the species and between varieties has also been reported.

A comparative study among the accessions is needed in order to determine the superiority of the species. This work is necessitated because this is going to be useful to the farmers, cultivars and entrepreneurs to select superior species. Hence the present investigation aims at the following.

- To analyze the impact of environmental factors on genetic diversity of chosen medicinal plants.
- Analysis of intra specific variations through RAPD - PCR fingerprint.
- Identification of superior genotypes in each species based on molecular pattern.